ERRATUM

In article on Biosynthesis of Phospholipid, by A. D. Welch, 1936, **35**, 107, line 5 should read "in the *depancreatized dog* on a lean meat sucrose diet."

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8913 P*

Cultivation of the Virus of the Common Cold in the Chorio-Allantoic Membrane of the Chick Embryo.

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We have reported the successful cultivation of the virus of the common cold in an anaerobic medium containing chick-embryo tissue.[1, 2] Our purpose in the present communication is to describe the cultivation of this virus in the chorio-allantoic membrane of the developing chick-embryo.

This technic for the cultivation of the viruses of infectious diseases was developed by Woodruff and Goodpasture[3] for the propagation of fowl pox virus, and later succeessfully applied by them

* P represents a preliminary, C a complete manuscript.

1 Dochez, A. R., Mills, K. C., and Kneeland, Y., Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

2 Dochez, A. R., Mills, K. C., and Kneeland, Y., Jr., *J. Exp. Med.*, 1936, **63**, 559.

3 Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

and others to a number of different viruses. Wilson Smith[4] reported the cultivation of a mouse-passage strain of human influenza virus by this means. A more elaborate series of similar experiments has already been recorded by Burnet.[5]

Our own experiment was as follows: In September, 1936, a nasal washing was obtained from an individual with a typical acute head cold of less than 24 hours' duration. The washing was passed rapidly through a Seitz filter, and concentrated to one-fourth its original volume by vacuum distillation. Two-tenths cc. of this bacteria-free filtrate, almost immediately after its isolation from the human source, were inoculated into the chorio-allantoic membranes of 12-day chick-embryos according to the technic described by previous authors. At the end of 2 or 3 days the membranes were removed and ground up with non-toxic broth so as to make a suspension of approximately 20%; 0.2 cc. of this were inoculated into each of a second series of eggs. Similarly these membranes were ground up and inoculated into a third. It is noteworthy that the small opaque foci visible on the membranes and which Burnet[5] described as resulting from the cultivation of influenza virus were also observed by us. A 20% suspension of the third-passage membranes was used to test for the presence of active virus. Human volunteers were used and the technic of intranasal inoculation under conditions of strict quarantine has been previously described.[2] In the present experiment, 2 volunteers were employed for inoculation. They had been in quarantine for a week prior to the test, during which time they had received 2 inoculations of an old culture of the virus of common cold. This material proved to be non-infective. The intensity and duration of symptoms in Volunteer No. 125

TABLE I.

Volunteer No. 125.

Date—1936	Sept. 11 to Sept. 18	19	20	21	22	23
Nasal obstruction	0	++	±	++	±	±
Sneezing	0	+	±	±	0	0
Nasal discharge	0	+	+	++	+++	+
Cough	0	++	++	+++	++	+
Sore throat	0	+	+	+	±	0
Headache	0	0	0	0	0	0
Anorexia	0	0	±	±	0	0
Throat	pale	slightly red	red	edema		red

Inoculation on Sept. 18.

± = mild.
\+ = moderate.
++ = marked.
+++ = severe.

4 Smith, Wilson, *Brit. J. Exp. Path.*, 1935, **16**, 508.

5 Burnet, F. M., *Brit. J. Exp. Path.*, 1936, **17**, 282.

produced by inoculation of the third-passage chorio-allantoic membrane are shown in Table I.

Volunteer No. 124 began to cought at 9 P. M. on the evening of the day of inoculation. The next morning the cough was worse, and he had a considerable degree of watery coryza and a slight soreness of the throat. Symptoms increased in intensity on the day following, remained about the same for another 24 hours, and were considerably abated on discharge 5 days after inoculation. Almost the identical series of events occurred with Volunteer No. 125, except that in his case, cough was more marked at the onset, and coryzal symptoms did not reach their maximum until 4 days after inoculation. Both men declared they had experienced head colds of full average severity; no constitutional symptoms of the influenzal type, such as general malaise and fever, were noted.

In summary, then, freshly obtained virus-containing material from a human cold was implanted on the chorio-allantoic membrane of the developing chick-embryo and passed through a series of 3 eggs. Material from the third series of eggs when tested on 2 human volunteers by intranasal inoculation produced in each instance a typical experimental cold. Dilution of the original material inoculated was so great that it seems unlikely that infection was due to the survival of a sufficient amount in the active state.

8914 C

Immunization in Rats Against Trichinella Spiralis.

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Attempts were made to immunize 35 white rats against *Trichinella spiralis* by way of the mouth.

These rats were formed into 3 groups, fed respectively on (1) trichina antiserum, (2) well-ground dehydrated trichina powder and (3) consecutive feedings of increasing doses of infested meat. A fourth group of 12 rats were injected intraperitoneally with varying amounts of Coca's alkaline suspension of trichina powder, as another possible method of protection against otherwise lethal doses of trichinous meat. Simultaneously, a series of normal rats were

used as controls. A total white blood count and differential were made in each of the 47 rats every second day, and symptoms were observed and recorded.

In Group One, each 3-month-old rat was fed from 6 to 9 cc. of rabbit antiserum (titer 1-10,000) and convalescent serum from trichinous hogs (negative titer) 24 hours before feeding them with the infested meat. Neither serum gave protection; rats died as early as the sixth and as late as the sixtieth day after infestation, all, including the normal serum controls, suffering from severe symptoms. No rat that died before the twentieth day after infestation showed an eosinophilic increase; 2 that lived for 60 days showed an eosinophilic count of 11% and 14%, which occurred on the thirty-second and the thirty-fourth day, respectively. Muscle larvae were found only in those rats that died after the twentieth day of infestation.

In Group Two, rats 3½ months old were fed with the fine dehydrated trichina powder over a period of 4 days before and for 20 days after feeding them with trichinous meat, which gave no protection. A lethal dose (5 gm. of meat with an average of 5 worms per low power field) was given to each rat, producing in each severe symptoms and death before the forty-fourth day. In each rat living over the eleventh day, an eosinophilia of from 5% to 17% was observed. Muscle larvae (from 5 to 30 per field) were present in both powder-fed and normal rats.

Group Three, consisting of rats 3 months old, was fed every fifth day with increasing doses of trichinous meat, and evidenced protection against large lethal doses fed later.[1] After a 10-day period, in which 2 doses of 0.5 gm. of infested meat were administered, mild symptoms were observed. Following a third feeding of one gm. (6 worms per field), a mild diarrhea was noted; however, in subsequent feedings of from 2 to 10 gm. (5 to 15 worms per field) no symptoms appeared. In this group only one rat died; all others lived through repeated feedings of infested meat, even though the last feeding of 10 gm. (15 worms per field) is twice the lethal dose for normal rats. In this group the eosinophiles rose as high as 16% on the thirtieth day after the first infestation. The average number of larvae found in the muscles of rats killed some 60 days after infestation was one-twentieth to one-half worm per field. This was very low, considering the large numbeer of worms experimentally administered.

In Group Four, intraperitoneal injections of 0.5 cc. and one cc. of Coca's suspension of trichina powder were no safeguard against

[1] McCoy, O. R., *Am. J. Hyg.*, 1931, **14**, 484.

lethal doses of infested meat.[2] All rats showed severe symptoms and died before the seventh day of the experiment.

In all the infested rats that survived the infestation, there was a marked increase in eosinophiles and a rapid rise in neutrophiles during the intestinal stage of the parasites.

Our observations record that the only protection given to rats for a limited period of time against infestations of *Trichinella spiralis* was the feeding of small and gradually increased doses of trichinous meat.[3] Attempts to protect rats by feeding anti- and convalescent serums, trichina powder, and by injecting intraperitoneally Coca's suspension of the dried and finely ground larvae failed to give any protection.

8915 C

Plant Extracts in the Nutrition of Guinea Pigs and Rabbits.*

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It has been known for years[1] that rabbits develop paralysis and soon die when they are restricted to rations of the concentrates commonly employed in livestock feeding. If, however, these rations are supplemented with good quality forage such as alfalfa hay they become entirely adequate. Our first basal ration, No. 800, simulated in composition a similar ration which contains 10% of alfalfa meal, and which had proved adequate for growth. It has the following composition:

Ground oats	53.1	Casein	0.25
Whole milk powder	34.2	Corn starch	0.43
Cod liver oil	1.8	Lard	0.04
Sodium chloride	0.9	Cellulose	0.14
		Salts	0.14

A long series of feedstuffs other than forages was studied in an

2 Lucker, J. T., *J. Parasit.*, 1932, **19**, 243.

3 Ducas, R., 1921, L'Immunité dans La Trichinose. Thése, Paris (Jouve et Cie), Pp. 47.

* Contribution from the Departments of Animal Husbandry and Agricultural Chemistry of the Missouri Agricultural Experiment Station. Journal Series No. 477.

1 Hogan A. G., and Ritchie, W. S., *Mo. Agr. Exp. Sta. Res. Bul.*, 1934, No. 219.

effort to find a soluble supplement that would make Ration 800 adequate for growth, but the only ones that offered any promise were some of the vegetable oils. The first successful ration employed, No. 2003, was made up of Ration 800 90 parts, and corn oil (Mazola) 10 parts. The data summarized in Table I indicate that this ration makes a close approach to complete adequacy during the growing period, but it was not entirely satisfactory.

TABLE I.
History of Rabbits That Received Ration 2003 Containing Corn Oil.

	Rabbit No.				
	♀ 426	♀ 430	♂ 433	♂ 443	♂ 447
Date	gm.	gm.	gm.	gm.	gm.
7-20-34	470	480			
11-20	2890	2640			
12-20	litter[1]				
1-1		*3170*[2]			
1-15-35	3220		445	490	480
3-5			1290	1210	1330
3-19			1730	1590	1050
3-26	*3510*[2]		1680	1800	1160
3-28			dead[3]		
4-11					dead[4]
5-14				*2550*[2]	

[1]Litter of 5, dead at birth.
[2]Ration changed.
[3]Pneumonia.
[4]Coccidiosis.

Madsen[2] reports that cottonseed oil confers partial protection from muscular dystrophy in guinea pigs and rabbits. Goettsch and Pappenheimer[3] observed that corn oil, cottonseed oil, peanut oil, and soy bean oil are all effective in preventing nutritional encephalomalacia in chicks. In addition to corn oil we have also tried soy bean oil, but in our experience wheat germ oil is by far the most potent. Even advanced cases of muscular dystrophy are healed by including it in the ration. The basal diet finally chosen is made up as follows: Ground oats 60, skimmilk powder 33, wheat germ oil 4, cod liver oil 1, NaCl 1, $CaCO_3$ 1. This ration invariably supports a rapid rate of growth, but it is grossly inadequate during the reproductive stage. Guinea pigs had very irregular oestrus cycles, few became pregnant, and some of these aborted. Only one litter was reared and it grew very slowly before weaning. The rabbits were less susceptible to these disturbances. In all 44 litters were obtained from females on various modifications of the basal diets,

[2] Madsen, L. L., *J. Nutr.*, 1936, **11**, 471.
[3] Goettsch, Marianne, and Pappenheimer, A. M., *J. Biol. Chem.*, 1936, **114**, 673.

but few survived. Seven litters of 2 each, and 4 litters of one each were reared. Post mortem examination of the young revealed two types of abnormalities, either of which would be sufficient explanation of the mortalities. In many cases there was liver damage, ranging from slight to severe. In extreme cases this organ was greatly enlarged, fatty, and gray in color. The most striking lesion, however, was extensive hemorrhage, which may occur in one or more sites such as the stomach, intestines, muscles, peritoneal cavity, or in the subcutaneous region. Many of the young that survived had recovered from obvious subcutaneous hemorrhages.

At this stage no supplement studied, except some form of forage, had offered any promise so we turned to a plant extract that had proved effective in somewhat similar abnormalities with suckling pigs (Mo. circ. 187, 1935). Young, vigorously growing barley was finely macerated, the juice expressed as completely as possible, and this was offered, instead of water, to a guinea pig. The remainder of the ration, No. 2178, was of the simplified type similar to that described in a previous publication. This animal reared a litter of 2 vigorous, rapidly growing young. Additional data appear in Table II. Subsequently similar extracts were concentrated *in vacuo* and incorporated in the ration at levels of 5 and 10%. The 5% level was obviously inadequate for guinea pigs as none of them produced young. One litter was reared on the 10% level, but additional observations are required for a satisfactory evaluation of the ration. One rabbit litter on each level was reared, but the minimum amount necessary to make the ration complete was not determined.

An effort was then made to obtain active fractions from forages. However, these studies have been retarded by the high summer temperatures, for it is impossible to distinguish between the failures that are due to unfavorable temperatures and those that are due to inadequate rations. The preparations that have given most promise up to the present are a dilute alcohol extract of the juice of young cereal grasses, oats or barley, and an ethyl ether extract of dehydrated alfalfa. Since guinea pigs and rabbits respond somewhat differently to the experimental diets they will be described separately.

With the alcohol extract alone advanced pregnancy in the guinea pigs was rarely observed and there was some evidence of early abortion. When normal litters were cast the mortality was low but growth was subnormal. With the ether extract alone the guinea pigs had regular oestrus cycles and became pregnant, but the ration was still inadequate, as of 5 females 3 died after giving birth to litters. Of the other 2, one gave birth to an apparently normal

TABLE II.

Plant Extracts Permit Guinea Pigs and Rabbits to Rear Litters.

Supplement	Animal No.	Litter No.	Weight	Weaning No.	Weight	Age, days	Remarks
				Guinea Pigs			
1	115	2	100, 95	2	194, 191	12	Good
3	137	3	124, 104, 103	3	191, 190, 189	13-15	,,
4, 5	151	3	104, 98, 88	3	190, 190, 181	22	,,
4, 5	167	3	104, 98, 87	3	190, 181, 171	18-23	,,
4	134 died	2	90, 89	2	131, 120	6	Milk failed
4	166 died	2	79, 76	1	138	11	,, ,,
4	168 died						Abortion
4	172	2	105, 90	1	190	21	Milk failed
4	147	1	137				Dead when found
5	181	3	93, 90, 88	2	100, 93	21	Excessive heat
6	138	2	89, 83	1	150	20	Poor lactation
6	138	2	102, 95	2	184, 108	17-19	,, ,,
6	189	2	115, 101	2	194, 187	18-19	,, ,,
				Rabbits			
2	474	7		6	405-465	30-41	
3	550	9		3	467-480	22-25	
4, 5	459	7		4	459-490	21-25	
4, 5	557 died	13		6			
4, 5	453	6		3	442-460	25-26	Coccidiosis
4, 5	562	9		3	420-453	28-31	
4	466	7		7	440-470	28-32	
4	567	5		0			
4	547	5		0			Suffocated oedema at birth
5	455	6		3	450-460	21-23	Did not accept young but had milk
5	459	4		0			No hemorrhages, suffocated by placental membranes
5	561	7		1			Hemorrhagic
6	532	8		1			,,
6	532	5		0			,,
							,,

young, but it was dead when found. The other female delivered 2 normal young but they grew slowly and only one survived. The ether extract seemed especially essential during pregnancy, and the alcohol extract seemed essential during lactation. Either fraction improved the basal diet, but in the amounts tested neither has been satisfactory by itself.

Insufficient data are available now to show whether or not the dilute alcohol extract improved the ration for rabbits in any significant way. When this preparation was included rabbits gave birth to the normal number of young, and of litters, but few were reared. Many were hemorrhagic and dead at birth. Practically all of those alive developed hemorrhages during the suckling stage and died. This condition has been observed repeatedly, and there is no doubt that it can be reproduced consistently. With the ether extract alone the rabbits delivered their young as usual, but as shown in Table II they were not very successful in rearing them. The point to be emphasized is, the ether extract is completely effective in preventing hemorrhages.

Combinations of the two extracts have been much more effective than either one alone. On rations that contained 2% of the alcohol extract and 1% of the ether extract, 2 guinea pigs and 3 rabbits have reared litters, though it may be that these rations do not provide optimum nutritional conditions.

Our observations on forage extracts are summarized in Table II. The supplements used are as follows:

1. Plant juice, *ad lib.*
2. Concentrated plant juice, dry matter 5% of ration.
3. Same as 2, dry matter 10% of ration.
4. Ether extract of dehydrated alfalfa, 1% of ration.
5. Plant juice soluble in 25% alcohol, dry matter 2% of ration.
6. Dilute alcohol extract of dehydrated alfalfa, 2% of ration.

8916 P

Observations on the Conjugated Oestrogens in the Urine of Pregnant Mares.

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Recently in this laboratory it was shown[1, 2] that during the last few days of pregnancy in women, immediately before and during labor, there occurs a rapid fall in the total amount of oestrogen excreted, simultaneously with a very great increase in the ratio of "free" ether-soluble oestrogen to conjugated ether-insoluble oestrogen. It was tentatively suggested that this conversion of the less physiologically potent conjugated hormones to the free hormones might be a factor in the initiation of labor. In order to obtain further evidence to support this theory, attempts were made to carry out similar investigations on other species, so that the research could be extended along more experimental lines. In this paper we report the results of colorimetric determinations[3] of the free and conjugated oestrogen in the urine of several mares at different stages of pregnancy. Previous work from this laboratory and elsewhere[4, 5, 6] has shown that the colorimetric procedure is applicable to pregnant mares' urine.

The estimations were carried out as follows: 100 cc. of fresh urine were adjusted to pH 6.0 after saturation with NaCl, and extracted several times with toluene. The combined toluene extracts were washed with saturated aqueous Na_2CO_3 and then extracted repeatedly with N. NaOH. The NaOH extract was made faintly acid with HCl, then alkaline with Na_2CO_3 and finally extracted with ether. The ethereal extract after washing with water was evaporated to dryness and the "free" oestrogen estimated colorimetrically in an aliquot portion of the residue. The oestrogen present in such a fraction is presumably a mixture of oestrone, equilin, hippulin, equilenin and oestradiol. At the present time no methods for the quantitative separation of these oestrogens from one another

1 Cohen, S. L., Marrian, G. F., and Watson, M., *Lancet*, 1935, **1**, 674.

2 Marrian, G. F., Cohen, S. L., and Watson, M., *J. Biol. Chem.*, 1935, **109**, lix.

3 Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1934, **28**, 1603.

4 Beall, D., and Marrian, G. F., *J. Soc. Chem. Ind.*, 1934.

5 Beall, D., and Edson, M., *Biochem. J.*, 1936, **30**, 577.

6 Cartland, C. F., Meyer, R. K., Miller, L. C., and Rutz, M. H., *J. Biol. Chem.*, 1935, **109**, 213.

are available and few data are available on their relative chromogenic powers. Of necessity, therefore, the colorimetric readings have been evaluated in terms of mg. of oestrone. Since the latter is the chief constituent of the mixture of oestrogens in mares' urine, this procedure is considered to be justifiable.

Attempts were made to determine the total oestrogen (free + conjugated) by carrying out a similar procedure on a second sample of urine which had been allowed to stand at pH 1.0 at room temperature for 7 days. We have since had reason to suspect, however, that these conditions are not sufficiently drastic to hydrolyse the conjugated oestrogen completely, and it is doubtful if our figures for the total oestrogen have much quantitative significance. The figures for the conjugated oestrogen showed that the average excretion at about the seventh month was close to 10 mg. (as oestrone) per 100 cc. urine. Wide variations from day to day even in the same mare were observed however. It seems probable that these variations are largely due to the unsatisfactory conditions of hydrolysis of the urine which were employed. From the seventh month onward, the oestrogen content of the urine fell irregularly to about 1-3 mg. per 100 cc. at term. A similar decrease in oestrogen content of the urine during the latter half of pregnancy has been previously reported by other authors.[5, 7, 8] We have not been successful in demonstrating a sudden fall in oestrogen excretion immediately before parturition. However, it is probable that if such a fall did occur, it would have been obscured by the wide day to day variations.

The figures for the "free" oestrogen generally varied between 0.2 and 0.5 mg. per 100 cc. although occasional variations from 0.04 to 1.35 mg. were encountered. These occasional high figures are possibly due to slight bacterial decomposition of the urine. There was no evidence of any regular progressive change in the amount of "free" oestrogen excreted during the last few months of pregnancy. In several instances we examined urine samples obtained from mares the day before and the day after foaling. The figures obtained for "free" oestrogen did not in any instance vary widely from the average excretion during the preceding few months.

We conclude from these experiments that parturition in the mare is not accompanied by marked changes in the ratio of "free' to conjugated oestrogen such as are observed in the human. If qualitatively similar changes do occur they are too small to be detected by the methods of estimation which we have employed.

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[7] Cole, H. H., and Saunders, F. J., *Endocrin.*, 1935, **19**, 199.

[8] Kober, S., *Klin. Woch.*, 1935, **14**, 381.

Our attempts to isolate the conjugated oestrogens in mare's urine have not so far been successful, but since we have obtained evidence on their probable chemical nature, the opportunity is taken to present a preliminary report on the work.

Distribution between various immiscible solvents of an alkali-washed butanol extract of urine from a mare at the seventh month of pregnancy, yielded a white amorphous solid containing about 40% of chromogenic oestrogen (calculated as oestrone). This material was insoluble in ether but easily soluble in water. It gave a negative naphthoresorcinol test indicating the absence of glucuronic acid. Sulphur was present however, and since after hydrolysis with dilute HCl a positive test for inorganic SO_4 was obtained it is possible that the oestrogens are conjugated with sulphuric acid. Millon's test was negative, indicating the blocking of the phenolic hydroxyl of the oestrogen by the conjugating group. Since the preparations obtained were obviously impure, a final decision concerning the chemical nature of these conjugated oestrogens must be deferred.

We wish to acknowledge with thanks the helpful cooperation of Professor N. E. McKinnon and Dr. R. D. H. Heard of the Connaught Laboratories in supplying us with samples of mares' urine. We are also indebted to the Banting Research Foundation for a personal grant to one of us (B.S.).

8917 P

Determination of Reduced Ascorbic Acid in Blood.

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Since the appearance of the clinical methods for determining blood ascorbic acid by Farmer and Abt[1, 2] considerable interest has been aroused in the study of vitamin C in various diseased conditions. The determination depends on the following proceduure: Deproteinization of the plasma by 10% metaphoric acid[3] and titra-

[1] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

[2] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.

[3] Fujita, A., and Iwatake, D., *Biochem. Z.*, 1935, **277**, 293.

tion of the reduced ascorbic acid present in the filtrate with 2:6 dichlorophenol indophenol.[4]

By the use of this method we have obtained high and low ascorbic acid values in presumably normal subjects, or at least in individuals in whom there was no reason to suspect vitamin C deficiency. Furthermore, determinations on the same subject within 2 or 3 hours yielded various blood ascorbic acid values. In all these determinations great care was exercised to avoid the presence of disturbing catalysts.[5]

Duplicate and triplicate readings on the sample of plasma by the same investigator or by another, yielded diverse amounts of vitamin C present. Naturally, this difficulty aroused our suspicion as to the accuracy of the method for clinical purposes. Our first thought was that the error lay in observing the true endpoint of the titration due to the oxidation of the dye. Titrations were then carried out by the use of a photometer and the pink endpoint brought to the same intensity in all cases. Even this failed to yield consistent figures where the plasma had been collected some time previous to the experiment. We particularly noticed that samples of blood plasma which gave certain readings at one time, failed to give the same readings 2 or 3 hours later. The common practice of keeping blood, either in the ice-chest or at room-temperature, for various lengths of time was investigated. Two hundred cubic centimeters of blood were withdrawn from a normal individual, oxalated, and plasma separated from the cells. The plasma was divided into 2 portions, one maintained at 26°C. and the other at 0°-5°C. Titrations were carried out hourly, and on a subsequent occasion the experiment was carried out at 10- to 15-minute intervals for 2 hours. In each instance a fresh solution of 10% metaphosphoric acid was carefully made up. The dye-values were repeatedly checked with distilled water and acetic acid for the blank readings. The actual titrations were started within one-half hour from the time the blood was drawn from the patient.

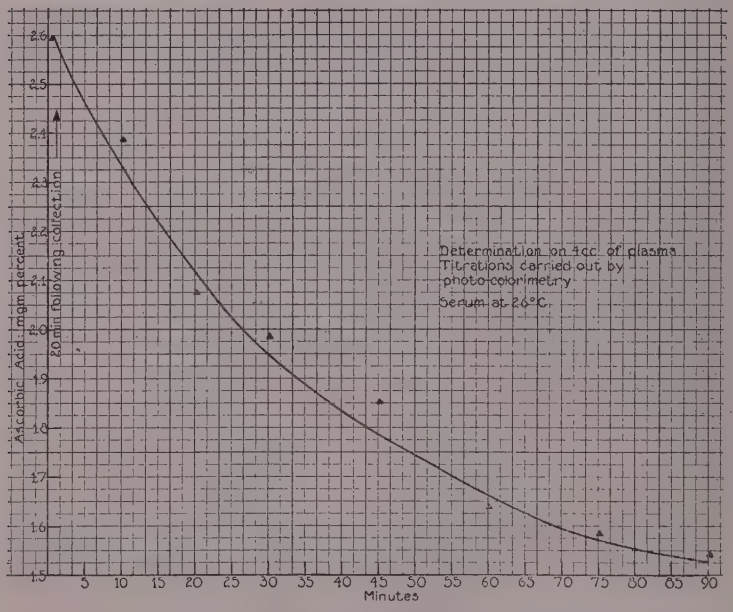
Four cc.* of plasma were diluted with 8 cc. of double-distilled water; to this was added 8 cc. of 10% metaphosphoric acid. Thorough mixing was effected by the use of a clean, dry stirring rod. The resulting precipitate was filtered through a Whatman

[4] Cohen, B., Gibbs, H. D., and Clark, W. M., *Public Health Rep. U. S. P. H. S.*, 1924, **39**, 804.

[5] Barron, E. S. G., Demeio, R. H., and Klemperer, F., *J. Biol. Chem.*, 1936, **112**, 625.

* 2 cc. of plasma were used, also, with 4 cc. of distilled water and 4 cc. of 10% metaphosphoric acid.

No. 42 filter paper. 8 cc. of the clean filtrate was used for titration with 2:6 dichlorophenol indophenol, which had been standardized so that 20 mg. of dye were equivalent to 20 mg. of vitamin C. A solution of 29 mg. of 2:6 dichlorophenol indophenol in 100 cc. of double distilled water is suitable for such purposes. The titrations were carried out by actual visual perception of the pink endpoint, and also by the use of the Evelyn colorimeter.[6] In no instance were metal parts, rubber, or cork material allowed to come in contact with the solution.



Plasma ascorbic acid values, in relation to the time of standing at 26°C. are shown in Chart 1. The loss of titratable ascorbic acid, presumably due to auto-oxidation, is even greater if the serum is kept standing at 0-5°C.

Summary. The ascorbic acid value of blood is materially affected by standing either in the ice-box or at room temperature. Determinations should be carried out within one-half hour after the collection of the blood.

[6] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, No. 1.

8918 C

The Gonad-Stimulating Potency of the Pars Anterior in Normal and Castrated Newts.

A. Elizabeth Adams and Virginia Mayo.

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Few experiments have been done which give evidence concerning differences in gonad-stimulating potency of the amphibian pituitary of the two sexes, either in the normal animal or after castration. Although Bardeen[1] reported no difference in pituitaries of males and females of *Rana pipiens,* Rugh[2] discovered that in this species the pituitary of the female is twice as potent as that of the male, and Rostand[3] has found a similar condition in *Rana temporaria* and *R. esculenta.* Using toad material *(Bufo arenarum),* Houssay, Giusti and Lascano-Gonzalez[4] observed no marked variation in potency of the pituitaries of the normal male, castrated male, normal female, and spawning female, and Novelli[5] was unable to demonstrate any difference in pituitaries of males of this species castrated for 30, 60, and 90 days as compared with those of the normal animal.

The criterion by which potency has usually been estimated is the ovulation (or egg-laying) induced in females in the non-breeding season judged by (1) the number of animals ovulating (or depositing eggs) in a definite time or (2) the number of grafts necessary to cause ovulation (or egg-laying) in a series consisting of a definite number of animals. The speed of the extra-seasonal ovulatory response is dependent on (1) the temperature[6] and (2) the period in the interbreeding season in which the tests are being made.[7]

Using *Triturus viridescens* in the non-breeding season (October through early March), the gonad-stimulating potency of anterior pituitaries of normal males and females and of those castrated for 4 days (Series II), 4 weeks (Series I) and 95 to 100 days (Series

1 Bardeen, H. W., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 846.

2 Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

3 Rostand, J., *Compt. rend. Soc. biol.*, 1935, **120**, 336.

4 Houssay, B,. Giusti, L., and Lascano-Gonzalez, J. M., *Compt. rend. Soc. biol.*, 1929, **102**, 864.

5 Novelli, A., *Compt. rend. Soc. biol.*, 1932, **111**, 476.

6 Bellerby, C. W., *Biochem. J.*, 1933, **27**, 2022; Rugh, R., *J. Exp. Zool.*, 1935, **71**, 149.

7 Rugh, R., *Biol. Bull.*, 1934, **66**, 22; *J. Exp. Zool.*, 1935, **71**, 149; Rostand, J., *Compt. rend. Soc. biol.*, 1935, **120**, 336.

TABLE I.

Number of daily homoplastic transplants of pars anterior from normal and castrated *Triturus viridescens* necessary to induce egg-laying in normal females in the non-breeding season. Fifteen hosts were used in every test except in Series I where twelve received grafts from castrated males.

No. of P.A. grafts to induce egg-laying	Number of Hosts Laying Eggs after Grafts, in 3 Series. Series II				Series I				Series III				Series II, I, III			
	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†
2	4	2	5	1									4	2	5	1
3	3	5	4	7					5	1	1	2	8	6	5	9
4	3	5	3	3	6	6	6	7	4	4	6	7	13	15	15	17
5	3	3	2	3		3	4	2	2	6	7	5	5	12	13	10
6					2	2	2	1	3	2	1		5	4	3	1
7	1				2	1			1				4	1		
8					1			1		1		1	1	1		2
9							1	1							1	1
10			1	1											1	1
11	1				2	1							3	1		
12						1								1		
13																
14					2	1							2	1		
15							1								1	
16							1			1				1	1	
Total No. hosts	15	15	15	15	15	15	15	12	15	15	15	15	45	45	45	42
Total No. P.A. grafted	62	54	54	60	108	95	96	61	66	85	68	67	236	234	218	188
Av. No. P.A. grafted per animal in Series	4.1	3.6	3.6	4.0	7.2	6.4	6.3	5.0	4.4	5.7 (4.9)*	4.5	4.5	5.24	5.2	4.84	4.48

*Average if newt receiving 16 P.A. is omitted.

†Castrated.

TABLE II.

Number of newts laying eggs after 5 and 6 daily homoplastic transplants of pars anterior from normal and castrated *Triturus viridescens*. Fifteen hosts were used in every test except in Series I where twelve received grafts from castrated males.

		No. of Hosts Laying Eggs after Grafts.															
		Series II				Series I				Series III				Series II, I, III			
		♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†
5 P.A.	No.	13	15	14	14	6	9	10	9	11	11	14	14	30	35	38	37
	%	86	100	93	93	40	60	66	75	73	73	93	93	67	78	84	88
6 P.A.	No.	13	15	14	14	8	11	12	10	14	13	15	14	35	39	41	38
	%	86	100	93	93	53	73	80	83	93	86	100	93	78	87	91	90.5

†Castrated.

III) was judged by the average number of grafts (one P.A. daily, intramuscularly) necessary to cause egg-laying in a series of 15 normal females (Table I). In Series II, the newts were gonadectomized between November 24 and December 3 and their pituitaries transplanted between November 28 and December 8 at 20°C. In Series I, castrations were performed between September 21 and October 6 and transplants made between October 30 and November 14 at 14°C. In Series III, the gonads were removed between November 15 and December 4 and grafting occurred between February 15 and March 2 at 20°C. The data were also analyzed on the basis of the number of newts in each group and the total number in all 3 groups that had laid eggs on the sixth or seventh days, *i. e.*, after receiving 5 or 6 pars anteriors (Table II).

It is evident (Table I) that there are no consistent or large differences in the ovulation-inducing potencies of pituitaries of castrated and normal newts as judged by the average number of glands necessary to induce ovulation in a series of 15 hosts. Only in Series I and in the combined data of the three series is the trend similar to that found in birds and mammals where the pituitaries of castrates are more potent than those of normal animals[8] and the pituitaries of normal males more potent than those of normal females.[8] In Series I some of the differences are also statistically significant, *e. g.*, castrated male compared with all the other groups and castrated female compared with normal female (barely significant), but this is not true in the combined data.

It is obvious from the table that there are usually one or two refractory hosts that fail to lay eggs until many pituitaries have been transplanted and this increases the average. For this reason, the number and percentage of the series of newts laying eggs after a certain number of grafts were determined. Using this standard (Table II) the data again show the *tendency* of pituitaries of castrates to be somewhat more potent than those of normals (not true of castrated males of Series II) and those of normal males to be slightly more potent than those of females (Series III is an exception). However, neither the differences in average number of pituitaries necessary to induce egg-laying in a series of animals nor those in the number of animals laying eggs after a certain number of grafts are sufficiently large to prove beyond question that significant

8 Allen, E., Edit., Sex and Internal Secretions, 1932; Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 308, 310; Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101; Evans, H. M., and Simpson, M. F., *Am. J. Physiol.*, 1929, **89**, 371, 375; Severinghaus, A. E., *Am. J. Physiol.*, 1932, **101**, 309; Smith, P. E., Severinghaus, A. E., and Leonard, S. L., *Anat. Rec.*, 1933, **57**, 177.

variations in gonad-stimulating potency exist in the pituitaries of castrated as compared with normal newts and of males as compared with females. If all of the series had been killed after five or six grafts, thus utilizing the criterion of ovulation rather than egg-laying, the data might have been more conclusive. It is also possible that gonadectomy in the fall of the year when the gonads are already mature may have relatively little effect on the gonad-stimulating hormonal content of the pituitaries as compared with possible effects in the summer when active spermatogenesis and oogenesis are in progress. This should be determined. But even in the fall, the already matured gonad is dependent for maintenance on the pituitary as the effects of hypophysectomy indicate, so that some gonad-stimulating hormone must be present and being released.

One other point emerges from an examination of the tables, namely that temperature affects the ovulatory reaction, for at 20°C., egg-laying occurred earlier, *i. e.*, after a smaller number of grafted pituitaries, than at 14°C. This is in agreement with the findings of Bellerby and Rugh.[9]

8919 C

Precipitation and Color Reaction for Ascorbic Acid: Specificity of Acidified Sodium Selenite Solution.

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Certain compounds of selenium, notably selenious acid and its soluble salts, undergo reduction with the formation of free selenium, which appears as a brick-red precipitate or a brick-red or orange-colored colloidal solution. The smaller the quantity of reducing agent, the more the likelihood of the liberated selenium being sufficiently dispersed to form the colloidal state.

The organic compounds of biological significance which display reducing properties are aldehydes, ketones, carbohydrates with a free carbonyl group, polyphenols, thio compounds, including cysteine and glutathione, and ascorbic acid. The thio compounds reduce more readily than carbohydrates with a free carbonyl group, and ascorbic acid may reduce even more readily than thio com-

[9] See footnote 6.

pounds. Ascorbic acid reduces even in the cold Fehling solution, alkaline or neutral solutions of silver salts, and potassium permanganate.[1]

To test the reducing action of ascorbic acid we have made a number of experiments with various compounds of selenium (selenic acid, sodium selenate, selenious acid and sodium selenite).

Ascorbic acid does not reduce in the cold or after heating on the water-bath either a 5% solution of selenic acid of sp. gr. 1.4 or crystalline sodium selenate solution (2%). When, however, the selenic acid is acidified with hydrochloric acid, reduction by ascorbic acid takes place gradually after heating or after prolonged standing. An acidified solution of sodium selenate (100 cc. of 2% sodium selenate plus 20 cc. concentrated hydrochloric acid) is also reduced by ascorbic acid. Hydrochloric acid reduces the selenate ion (SeO_4^{--}) in selenic acid and in sodium selenate to the selenite ion (SeO_3^{--}). The selenite ion in the form of selenious acid or sodium selenite is easily reduced to free selenium by a variety of organic compounds.

Certain thio compounds reduce selenic acid directly. Thiacetic acid reduces selenic acid (5% solution of selenic acid, sp. gr. 1.4) in the cold but cysteine, thioglycollic acid and thiobarbituric acid on the application of heat. Thiosalicylic acid shows no reducing action.

We find that ascorbic acid reduces in the cold selenious acid (5% solution of selenium dioxide). Emmerie has made a similar observation.[2] We also find that thioglycollic acid, cysteine and glutathione reduce this reagent at room temperature, while thioacetic acid, thiobarbituric acid and thiosalicylic acid cause reduction of the selenious acid reagent only after heating. The 2 compounds lacking a free thio or sulphydryl group, cystine and methionine, reduce neither at room temperature nor on heating.

Ascorbic acid reduces sodium selenite to elemental selenium. To test the effect of ascorbic acid on sodium selenite 3 reagents were employed: Reagent A—2% sodium selenite solution; Reagent B—2% sodium selenite in 10% sodium carbonate solution; Reagent C—100 cc. of 2% sodium selenite solution plus 20 cc. concentrated hydrochloric acid.

We have reported in 1934 that ascorbic acid reduces all the 3 selenite reagents.[3] The thio compounds—ethyl mercaptan, o-thio-

[1] Szent-Györgi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] Emmerie, A., *Acta Brevia Neerland. Physiol. Pharm. Microbiol.*, 1934, **4**, 141.

[3] Levine, V. E., and Rosenthal, B. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1092.

cresol, α- and β-thionaphthol, thiosemicarbazide, thioacetic acid, thioglycollic acid, thiobarbituric acid, thiosalicylic acid, cysteine, and glutathione—also reduce the 3 selenite reagents. Ascorbic acid, however, reduces in the cold only the acidified selenite reagent (C), while the thio compounds require heat in order to reduce the same reagent. The thio compounds as well as ascorbic acid reduce in the cold the straight selenite reagent (A) and the alkalinized reagent (B).

Reduction of the selenite reagents is conveniently carried out by the interaction of 3 cc. of the reagent with 1 cc. of ascorbic acid solution or solution of thio compound. Due to the ease with which copper catalyzes the oxidation of ascorbic acid,[4] we have made up reagents as well as solutions of ascorbic acid with ordinary distilled water redistilled in glass. The water was thus freed from copper and iron. Copper was detected by McFarlane's method[5] using sodium diethyldithiocarbamate as the reagent described by Callan and Henderson.[6] The golden yellow color obtained in the presence of copper is extracted with amyl alcohol. Iron was detected by means of potassium thiocyanate, and the red color resulting is also extracted with amyl alcohol.

Ascorbic acid also reduces a solution of sodium tellurite to elemental tellurium, yielding a brownish black precipitate or a dark brown colloidal solution. The 3 reagents (A) 2% sodium tellurite solution; (B) 2% sodium tellurite in 10% sodium carbonate solution; (C) 100 cc. of 2% sodium tellurite solution plus 20 cc. concentrated hydrochloric acid are reduced in the cold by ascorbic acid. The alkalinized tellurite reagent is reduced by carbohydrates with a free carbonyl group. The 3 tellurite reagents are all reduced on heating by the thio compounds, o-thiocresol, ethyl mercaptan, thioacetic acid, thioglycollic acid, glutathione and cysteine. The acidified tellurite reagent is not as specific for ascorbic acid as the corresponding selenite reagent. Ethyl mercaptan and o-thiocresol reduce the acidified tellurite reagent in the cold, although thioacetic acid, thioglycollic acid, cysteine, and glutathione reduce this reagent only on heating.

The limit of sensitivity for ascorbic acid with reference to reduction in the cold of the acidified selenite reagent (C) is 0.04 mg. The limit of sensitivity with relation to the reduction on heating of the acidified selenite reagent is 0.01 mg. for o-thiocresol 0.025 mg.

[4] Mawson, C. O., *Biochem. J.*, 1933, **29**, 569.

[5] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[6] Callan, T., and Henderson, J. A. R., *Analyst*, 1929, **54**, 650.

for ethyl mercaptan, 0.015 mg. for thioacetic acid, 0.003 mg. for thioglycollic acid, 0.025 mg. for glutathione, and 0.05 mg. for cysteine.

Glucoreductones, made according to the method of Kertesz[7] and neutralized with dilute hydrochloric acid, reduce all the 3 selenite reagents, but only the alkalinized selenite reagent (B) is reduced in the cold. The straight selenite reagent (A) and the acidified selenite reagent (C) are reduced only after heating. The glucoreductones do not reduce the acidified selenite reagent in the cold, while ascorbic acid does.

Carbohydrates with a free carbonyl group reduce after heating the alkalinized selenite reagent (B).[8] They also reduce the straight selenite reagent (A), but not as vigorously or profusely. Acid depresses the reducing activity of the carbohydrates with a free carbonyl group. The carbohydrates that reduce sodium selenite solution or the alkalinized sodium selenite solution on heating are arabinose, rhamnose, xylose, glucose, glucosamine, fructose, galactose, mannose, melibiose, cellobiose, lactose and maltose. Other carbohydrates, sucrose, trehalose, raffinose, melezitose, cellulose, starch, dextrin, glycogen, inulin, glucosides, glycoproteins and glycolipins, reduce only after hydrolysis and subsequent neutralization.

Compounds other than reducing carbohydrates and thio compounds do not reduce the alkalinized solution of sodium selenite, but reduce the acidified solution. This reduction is accomplished only after heating. Among the compounds reacting with the acidified selenite reagent (C) on heating, we may mention formaldehyde, paraldehyde, acetyldehyde, acetone, ethyl acetoacetate, β-hydroxybutyric acid, lactic acid, pyruvic acid, malonic acid, mucic acid, pyrocatchin, resorcin, pyrogallol, hydroquinone, phloroglucin, adrenalin, homogentisic acid, and creatinine. Orcein reduces but not as profusely as resorcin. Alkaline solutions are not desirable for testing the reducing property of polyphenols with respect to sodium selenite, since a red, red brown or dark brown coloration develops as a result of the hastened oxidation in an alkaline medium.

Homogentisic acid reduces in the cold the straight sodium selenite reagent (A) and the alkalinized sodium selenite reagent (B). The alkaline reagent tends to darken the mixture containing homogentisic acid due to the rapid oxidation induced by the alkaline medium. Addition of sodium carbonate solution alone to a homogentisic acid

[7] Kertesz, Z. I., *J. Biol. Chem.*, 1934, **104**, 483.

[8] Levine, V. E., *Biochem. Bull*, 1915, **4**, 217.

solution causes the formation of red brown to black brown coloration which begins near the surface (proximity to oxygen). Homogentisic acid reduces the acidified selenite solution (C) poorly, but only after heating. Homogentisic acid (1 cc. containing 1 mg.) and 2 cc. of acidified selenite solution allowed to stand at room temperature showed faint reduction only at the end of 48 hours. Allantoin and uric acid do not reduce any of the selenite reagents at room temperature or at higher temperatures.

A brick red coloration is imparted in the cold to plant and animal tissues containing ascorbic acid when they are sprinkled with the acidified selenite reagent (C). We have observed this formation of the brick red color with lemon pulp, orange pulp, banana pulp, adrenal gland and liver.

Conclusion. Ascorbic acid reduces in the cold selenious acid, a straight sodium selenite solution, or one alkalinized or acidified. Reducing carbohydrates reduce only the alkalinized solution on heating. Thio compounds, including cysteine and glutathione, also reduce at room temperature the straight sodium selenite solution and the alkalinized solution, but the acidified solution only on the application of heat. A number of aldehydes, ketones, polyphenols, and creatinine also reduce the acidified selenite solution, but only on heating. Ascorbic acid differs from all the organic substances we have thus far tested, since it possesses the unique and specific property of reducing the acidified selenite reagent in the cold. The acidified sodium selenite reagent applied to plant and animal tissue rich in ascorbic acid is easily reduced in the cold with the formation of a brick red color characteristic of free selenium.

8920 P

Suppression of Persisting Corpora Lutea in Hypophysectomized Rats.*

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Although a great deal of information is available concerning the nature of the stimulus which causes a corpus luteum to form, little

* Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by Frederick L. Hisaw.

is known of the circumstances which determine its life span. Several observations made by different workers have served to indicate, however, that in the normal animal the involution of the corpus luteum is not a passive phenomenon. Especially noteworthy is the failure of the corpora lutea to regress following hysterectomy in guinea pigs (Loeb[1]) and after hypophysectomy in the adult rat (Smith[2]). Furthermore, the cessation of function and retrogression of the corpora lutea at or near the termination of an oestrous cycle or of a pregnant or pseudopregnant state makes it quite certain that this luteal failure is conditioned by extrinsic factors.

In the present investigation we determined the influence of several hormone preparations on the survival and histological structure of persisting corpora lutea in hypophysectomized rats. The plan of these experiments was to produce heavily luteinized ovaries in young adult rats by injecting crude pituitary extract, remove the hypophysis, allow time for follicular atresia and then replace individually those hormones whose presence in the blood stream was either lacking or not indicated. The corpora lutea were allowed to persist for 15 days before they were subjected to the influence of injected hormones. The injections were continued over 10 days. The crude pituitary extract given prior to the removal of the hypophysis produced ovaries which averaged 100 mg.[†] and contained numerous corpora lutea. On the 15th and 25th post-operative days the ovaries averaged 63 and 45 mg. respectively. These ovaries always contained numerous persisting corpora lutea and a few small follicles without antra.

The injection of 40 R.U. oestrin daily for 10 days did not significantly alter the weight (av. 42 mg.) or histological structure of the ovaries. The uterus and vagina showed marked oestrous changes.

Another group of 4 animals received 0.5 Rb.U. each daily of progestin. The average ovarian weight (53 mg.) was slightly above the average for the operated controls but the difference was probably not significant since the ovaries were not distinguishable histologically from the controls.

The number and appearance of the persisting corpora lutea was likewise not affected by the follicle stimulating fraction of the pituitary.

A sharp decrease in the size of the ovaries was found after treat-

[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1923, **20**, 441.

[2] Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

[†] In each case the average ovarian weight represents a group of not less than 5 animals.

ment with the luteinizing fraction (LH). The 11 animals in this group each received 3 to 5 mg. LH powder daily. The average ovarian weight for this group was 16 mg. (range, 10 to 23 mg.). Four separately prepared batches of LH were used on these animals with about equal success. In some cases the persisting corpora lutea could no longer be identified by gross examination at autopsy while in others a few degenerate appearing corpora remained. Microscopic study showed that extensive involution of the luteal tissue had occurred. The mechanism by which this involution is brought about is not clear but it appears to be due to a direct action on the corpora lutea. Whether this reaction can be attributed to the LH itself or to some closely allied substance contained in the LH preparation is not known.

Summary. The character of persisting corpora lutea in hypophysectomized rats was not influenced by the injection of oestrin, progestin or the follicle stimulating hormone of the hypophysis. The luteinizing fraction, however, caused almost total regression of the corpora lutea with marked diminution of ovarian weight.

8921 C

Effect of Isoartemisin on the Circulatory System.

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When santonin is administered to animals, a small amount of it is excreted in the urine in the form of oxysantonins. Jaffé[1] obtained a-oxysantonin and b-oxysantonin from the urine of dogs and rabbits, respectively, in this manner. Since the sterioisomeric oxides are apparently detoxification products of santonin, a comparative study of the pharmacological properties of isoartemisin or d-oxysantonin[2] (later called a-oxysantonin[3]) and of santonin was undertaken.

The action of isoartemisin on the frog heart has been studied by Trendelenburg.[4] The effect produced by perfusion of a 1:5000

[1] Jaffé, *Z. Physiol. Chem.*, 1897, **22**, 538.

[2] Wedekind and Tettweiler, *Ber.*, 1905, **38**, 1848.

[3] Wedekind and Tettweiler, *Ber.*, 1931, **64B**, 387.

[4] Trendelenburg, *Arch. Exp. Path. Pharmakol.*, 1915, **79**, 190.

solution was found to be somewhat weaker than that of santonin in equivalent concentration.

The effect of isoartemisin on the peripheral circulation has not been reported.

1. *Influence on Cardiac Rate, Amplitude of Contraction, and Output.* The method employed for perfusion of the frog heart was essentially the same as that described by Sollman and Barlow.[5] In addition, the right aorta was ligated as close to the heart as possible and the left aorta was cannulated close to the bifurcation.

After making a normal heart record and measuring the output per 5 minutes while perfusing Howell-Ringer solution, a saturated solution of isoartemisin in Howell-Ringer solution (80 mg. liter) was perfused. Five experiments were performed. Only Howell-Ringer solution was perfused in 5 control experiments.

Although there was an occasional decrease in amplitude and an irregularity of contraction when the isoartemisin solution was first perfused, the heart beat soon returned to normal. The cardiac output was not significantly affected over a 30-minute period.

2. *Effect on Peripheral Circulation.* The technic employed for perfusion of frog legs was that devised by Laewen[6] and improved by Trendelenburg.[7] Instead of injecting the saturated solution of isoartemisin into the perfusion cannula, however, it was thought preferable to use a Y-cannula and perfuse it separately. Isoartemisin caused an increase in rate of perfusion in all but 2 cases, as shown in Table I. It is believed that in these 2 cases not enough time was allowed for the rate of perfusion to become approximately constant. This is indicated by the sudden decrease in rate on returning to Howell-Ringer solution. The variable increase in rate of perfusion obtained with the 0.008% isoartemisin solution was probably due to the variable extent of the edema.

The vasodilator action of isoartemisin is a property of santonin which has not been lost through oxidation. Bertino[8] has shown that 1:5000 to 1:10,000 solutions of santonin in Ringer's solution have a marked dilator action when tested by the Laewen-Trendelenburg method. He also reports a further dilation on reperfusion of Ringer's solution after santonin. This was not observed after isoartemisin.

Conclusions. 1. Cardiac rate, amplitude of contraction, and output are not significantly affected by perfusion of an 0.008% solu-

[5] Sollman and Barlow, *J. Pharmacol.*, 1926, **29**, 233.

[6] Laewen, *Arch. Exp. Path. Pharmakol.*, 1904, **51**, 416.

[7] Trendelenburg, *Arch. Exp. Path. Pharmakol.*, 1910, **63**, 165.

[8] Bertino, *Arch. Farmacol. Sper.*, 1933, **55-56**, 579.

TABLE I.
Rate of Perfusion (drops/min.).

Exp. No.		Ringer Solution	Isoartemisin Solution	Change in Rate	% Change
1	a	29.3	29.2	—0.1	—
	b	21.0	22.8	1.8	8.6
2	a	20.8	22.9	2.1	10.1
	b	19.4	21.0	1.6	8.2
3	a	15.8	21.2	5.4	34.2
	b	11.6	15.8	4.2	36.2
4	a	13.1	14.2	1.1	8.4
	b	11.2	16.6	5.4	48.2
	c	13.0	17.4	4.4	33.8
5	a	21.4	23.3	1.9	8.9
6	a	30.6	30.2	—0.4	—
	b	23.3	27.0	3.7	15.9
	c	23.1	29.1	6.0	26.0
7	a	26.4	29.6	3.2	12.1
	b	26.0	27.6	1.6	6.2
	c	24.5	38.9	14.4	58.8
8	a	18.2	22.8	4.6	25.3
	b	16.2	17.4	1.2	7.4
9	a	30.2	35.9	5.7	18.9
	b	25.7	33.9	8.2	31.9
	c	18.8	23.8	5.0	26.6
10	a	30.2	33.3	3.1	10.3
	b	28.0	30.1	2.1	7.5
	c	22.8	25.5	2.7	11.8
11	a	17.7	24.7	7.0	39.6
	b	16.7	17.7	1.0	6.0

tion of isoartemisin. 2. A slight but significant dilator action on the vessels of the frog leg was observed.

8922 P

Differential Cell Counts of the Pituitary in the Thymus Treated Strain of Rats.

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In an attempt to ascertain the possible mechanism of the previously reported acceleration in the rate of growth and development of rats produced by injections of thymus extract, differential cell counts of the pituitary, in a series of rats have been made, at intervals between birth and 45 days of age, the period of most rapid growth.

All rats (test and controls) were killed with ether, the pituitaries

removed as soon as possible, weighed immediately, fixed in Helly's fluid embedded in paraffin and sectioned horizontally; interrupted serial sections were made and stained by a modified Mallory's stain. Slight overstaining emphasizes the acidophils and made counting easier. No attempt was made to record finer histologic differences in the cells, although all counts were made under oil-immersion with a Zeiss ocular net micrometer.

As far as possible, the same procedure was followed in making a count; one begins at one lateral aspect of the anterior lobe, as near the "equator" as possible and counts approximately 500 cells while moving toward the center of the gland; this is repeated from the opposite edge of the gland; about every 3rd oil immersion field is counted. A second section is then counted in the same manner, distant about 50 or more micra from the first in the smaller animals, farther in the larger. At least 2000 cells per pituitary per animal are reported; in two instances, once in the female thymus group and again in the male control, the percentage differences in acidophils betwen the first two sections counted were 10.1 and 10.5 respectively. In these two animals 2000 more cells were counted and the average of the total 4000 cells reported.

With these exceptions differences between consecutive counts in the same pituitary are quite uniform. In the thymus-injected group the average difference in percentage of acidophils was 2.4 (7.3-0.2) and 3.4 (10.1-0.5) and for the basophils 0.9 (4.3-0.1) and 1.2 (2.9-0.2) for males and females respectively; in the controls for acidophils it was 3.6 (10.5-0.2) and 3.6 (7.4-0.1), for basophils 0.8 (1.9-0.2) and 1.2 (2.5-0.1), respectively in males and females.

Table I shows the results of counts in 36 thymus-injected rats

TABLE I.

Pituitary cell counts in thymus-injected and control rats, male and female, in various age groups. Figures in parentheses indicate number of rats, per group, per age studied. * indicates that protocols from these rats are shown in Table IV.

Age Group	Males						Females					
	Thymus Injected			Control			Control			Thymus Injected		
	Acid.		Baso.	Acid.		Baso.	Acid.		Baso.	Acid.		Baso.
0-1	15.5	(2)	1.2	7.4	(2)	1.0	11.7	(2)	0.9	10.7	(3)	1.3
*4-7	23.6	(3)	3.9	14.2	(2)	1.1	11.6	(2)	2.4	18.6	(2)	4.4
*9-11	24.0	(3)	2.8	14.2	(1)	2.1	15.4	(1)	3.6	21.2	(2)	4.9*
13-17	25.1	(2)	2.2	24.2	(1)	2.7	19.3	(1)	4.9	20.8	(2)	5.6
19-21	21.2	(2)	2.6	27.4	(2)	5.1	23.6	(2)	7.7	18.0	(2)	6.1
25-27	26.7	(2)	6.6	24.8	(1)	4.6	16.9	(1)	8.8	28.3	(1)	3.4
29-32	27.5	(1)	5.4	21.7	(2)	4.7	29.1	(1)	2.1	23.5	(1)	4.9
33-37	24.3	(1)	1.4	26.9	(2)	5.4	27.8	(3)	7.7	25.3	(3)	3.7*
39-41	29.3	(1)	5.2	28.4	(2)	2.9	23.7	(2)	5.6	28.3	(1)	5.4
43-45	22.4	(1)	4.5	29.0	(1)	5.4	22.5	(1)	6.7	30.4	(1)	4.1
Total		(18)			(16)			(16)			(18)	

and 32 controls. Although the number of rats per age group is too few to permit tabulation of frequency distribution or calculation of mean and standard deviations, the percentage of acidophils seems definitely higher in the thymus-treated precocious strain between birth and about 15 days of age.

TABLE II.

Comparison of pituitary cell counts in male and female, thymus-injected and control rats, from birth to 13 days of age; and from 13-45 days. Numbers in parentheses, the same as for Table I.

Age Group	Males						Females					
	Thymus Injected			Control			Control			Thymus Injected		
	Acid.		Baso.	Acid.		Baso.	Acid.		Baso.	Acid.		Baso.
0-13	21.7	(8)	2.8	11.5	(5)	1.2	12.4	(5)	2.0	15.9	(7)	3.2
13-45	24.9	(10)	3.9	26.1	(11)	4.4	24.2	(11)	6.6	24.0	(11)	4.6

TABLE III.

Pituitary cell counts, thymus-injected and control rats, from birth to 13 days of age and from 13-45 days. Numbers in parentheses same as before.

Age Group	Thymus Injected			Total Cells Counted	Control		
	Acid.		Baso.		Acid.		Baso.
0-13	19.0	(15)	3.0	54,954	11.9	(10)	1.6
13-45	24.4	(21)	4.3	93,550	25.1	(22)	5.5

Tables II and III show this more clearly. From birth to 13 days of age in more rapidly growing thymus-injected rats the acidophil content is 60% higher than in the non-injected controls (19.0% against 11.9%). The same age group, in the more sexually precocious strain (thymus-injected), shows an 88% greater basophil count than the control (3.0% opposed to 1.6%).

TABLE IV.

Relationship between percentage of acidophils and body-weight (growth) in thymus-injected rats at different ages and weights. * and † indicate litter mates at different ages.

			Thymus Injected							
Sex	Gen.	Age	Wt. gms.	Ears	Teeth	Eyes	Hair	Genital	Acid.	Remarks
M*	F10	4	22	1	2	2	1	3	19.6	Well nourished
M	F10	5	28	1	1	2	1	3	27.9	
M	F4	7	29	1	4	5	3	—	23.3	
M	F7	9	54	0	1	2	2	3	33.9	
M	F10	10	36	1	1	2	2	4	23.3	
M*	F10	11	25	1	2	3	1	6	15.4	Poorly nourished
F†	F7	9	56	0	1	2	1	7	25.8	
F*	F10	11	30	1	2	2	1	—	16.6	
F	F7	34	88	1	1	2	1	14	31.6	
F†	F7	34	58	1	1	2	1	6	22.0	Poor growth after 14 days

TABLE V.

Relationship between percentage of acidophils and body weight (growth) in control rats. Note similarity of weight and percentage of acidophils, but at a greater age, to treated rats in Table IV.

		Control				
Sex	Age	Wt. gms.	% Acid	Remarks	General Development	
M	20	34	24.2	Better than normal	Ears	2½-3
M	21	47	30.4	Much larger than normal	Teeth	9-10
M	35	48	22.9	Below normal	Eyes	14-17
M	36	75	30.8	Very good weight	Hair	12-16
F	5	9	9.7	Less than normal	Genitals:	
F	5	12	13.4	Normal	Testes	31-40
F	20	28	18.0	"	Vagina	55-62
F	20	36	29.0	Better than normal		

The correlation between rapid growth in thymus-treated animals and acidophil content of the pituitary, suggested by the first 3 tables, seems further substantiated by Tables IV and V, in which body weight in grams, irrespective of age, seems to bear a definite relationship to the acidophil count in the pituitary in both control and thymus-treated rats.

Further work is in progress to ascertain the possible significance of these findings.

8923 C

Relation of Oestrin and Pregnancy Urine Hormone in Influencing Uterine Motility.*

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The relationship which exists between oestrin and progestin in regulating uterine motility suggested the possibility of a similar relation between oestrin and pregnancy urine hormone. Reynolds and Friedman[1] and Reynolds[2] have shown that the uterine motility of the unanesthetized rabbit, as recorded by the uterine fistula method, is inhibited by intravenous injections of pregnancy urine hormone. The inhibition of uterine motility induced by pregnancy urine hormone in either normal rabbits or castrated rabbits treated with oestrin, is somewhat similar to that obtained with progestin

* Aided by a grant from the Rockefeller Foundation.

1 Reynolds, S., and Friedman, M., *Am. J. Physiol.*, 1930, **94**, 705.

2 Reynolds, S., *Am. J. Physiol.*, 1932, **100**, 545.

and is characterized by either a disturbance in normal rhythm, a diminished height of contraction or complete quiescence. The inhibition is transitory in the castrated rabbit and the motility returns to normal usually within 24 hours. It will be shown that if a castrated female rabbit is given sufficient amounts of oestrin, the pregnancy urine hormone in doses ordinarily capable of inhibiting uterine activity, is unable to act.

In the first series of experiments, uterine fistulae were prepared, according to the technique of Reynolds,[3] in 12 adult female rabbits in heat and records of uterine motility were taken 3 to 4 days after the operation. Six to 9 hours after intravenous injections of varying doses of an extract of pregnancy urine (Follutin)† or other gonad stimulating urines, 11 of the 12 rabbits responded by complete inhibition of uterine motility, Table I. Ovulation occurred

TABLE I.

No. of Rabbits	Dose	Motility
6	6 to 200 R.U. Follutin	0
2	75 and 150 cc. equiv. Teratoma urine	0
2	100 cc. equiv. Castrate urine	0
1	75 cc. equiv. Menopause urine	0
1 (2) tests	50 and 100 cc. equiv. Menopause urine	3+

in all rabbits but one, in which a loss of activity of the hormone accounted for the failure of ovulation and inhibition of motility. These results confirm Reynolds.[2]

In the second series of experiments, 9 adult female rabbits were castrated and the uterine fistulae prepared. They were treated intermittently with oestrin‡ (in oil solution) subcutaneously for varying periods and then were injected intravenously with Follutin in doses from 10 to 500 R.U. In a series of 28 tests, it was found that 16 resulted in varying degrees of inhibition of uterine motility, but in 12 cases motility was not altered. Of the 16 positive cases, 7 showed complete inhibition and 9 showed only slight irregularities in rhythm or diminished height of contraction (1+ or 2+ motility). Protocols of several representative experiments are given in Table II.

It was noted that injections of Follutin or other gonad stimulating urine extracts consistently inhibited uterine motility to a

[3] Reynolds, S., *Am. J. Physiol.*, 1930, **92**, 420.

† The Follutin was obtained through the kindness of Dr. J. A. Morrell of Squibb & Co.

‡ The oestrin was obtained through the kindness of Dr. W. R. Bond of The Rare Chemical Corp.

TABLE II.

Rabbit 4. 1/16—Castrated. 1/19 to 1/25—Given 5 R.U. oest. per day. 1/25—Fistula prepared. 1/25 to 1/27—Given 10 R.U. oest. per day. 1/28—Good motility, given 10 R.U. Foll., resulted in 3+ motility. Given 10 R.U. oest., 1/29, good motility, given 40 R.U. Foll., resulted in 3+ motility.

Rabbit 6. 2/1—Fistula prepared. 2/17—Castrated. 2/17 to 2/20—Given 5 R.U. oest. daily. No oest. 2/21 to 2/25. 2/26—Good motility, given 10 R.U. Foll., motility 1+. 2/27 to 3/1—given 5 R.U. oest. daily. 3/2—Good motility, given 10 R.U. Foll., motility 3+. 3/3—Good motility, given 40 R.U. Foll., motility 3+.

Rabbit 9. 4/20—Fistula prepared. 4/27—Castrated, given 5 R.U. oest. 4/28—Good motility, given 60 R.U. Foll., motility 1+. 4/29—Given 5 R.U. oest. 4/30—Good motility, given 100 R.U. Foll., motility 0.

Rabbit 21. 7/31—Castrated. 8/3—Fistula prepared. 7/31 to 8/23 given 5 R.U. oest. daily. 8/24—Good motility, given 10 R.U. Foll., motility 3+.

0	motility	No activity in uterus.
1+	"	Slight motility, no rhythm.
2+	"	Moderate activity, some rhythm.
3+	"	Marked activity of pre-injection type.

more marked degree in the normal rabbit than in the oestrin-treated castrated rabbit. In the 11 positive cases in normal rabbits, including the 6 treated with Follutin, complete quiescence resulted. However, in the oestrin-treated castrated rabbits injected with comparable doses of Follutin, complete quiescence was obtained in only 7 of the 28 tests. Because of the relative ease in obtaining complete quiescence of uterine motility in the normal rabbit in heat with Follutin, it seems that possibly the presence of the ovaries may favor in some way, the inhibiting reaction. Reynolds[2] has suggested this possibility based on his own observations. Recently, Donnet[4] has shown that, in the normal rabbit, large doses of oestrin will cause the cessation of spontaneous uterine motility and loss of sensitivity to pituitrin.

Follutin failed to inhibit uterine motility in 12 tests out of 28 when oestrin-treated castrated rabbits were used. On examining the protocols of each rabbit, it was observed that whenever there was a failure of Follutin to inhibit uterine motility, the oestrin level was high or the amount of Follutin administered was relatively small. For example, in rabbit 6, Table II, it is seen that 10 R.U. of Follutin was sufficient at one time to inhibit motility, yet after further oestrin treatment, this amount of Follutin failed to do so. No quantitative studies were made relative to the amounts of oestrin necessary to negate the influence of Follutin because of the intermittent methods of giving oestrin in these experiments.

It is concluded, therefore, that oestrin, if present in sufficient quantities, can override the inhibitory action of pregnancy urine hormone on uterine motility in the castrated rabbit.

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[4] Donnet, V., *Comp. Rend. Soc. Biol.*, 1936, **121**, 65.

8924 P

Successful Treatment of Human Pellagra with the "Filtrate Factor."

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György,[1] Elvehjem and Koehn,[2] and Lepkovsky and Jukes[3, 4] have divided the vitamin B_2 complex into 3 components—flavins, the rat antiacrodynia (vitamin B_6) factor, and the filtrate or chick antidermatitis factor. Birch, György and Harris[5] concluded that the human "P-P" factor and the canine anti-blacktongue factor are different, both from the rat vitamin B_6 and from lactoflavin, and that the canine anti-blacktongue factor may be identical with the "P-P" factor. They found that pellagra- and blacktongue-producing diets were rich in vitamin B_6. Maize was especially rich. Dogs fed on a purified diet supplemented with vitamin B_1 and lactoflavin could not be cured of blacktongue unless both maize (rat vitamin B_6) and liver extract were added to the diet. Dann[6] likewise found that both yellow and white maize contained goodly amounts of vitamin B_6. Dann,[6] and Spies and Chinn, as quoted by Birch, *et al.*,[5] were unable to cure pellagrins with lactoflavin. The experimental evidence of Jukes and Lepkovsky[7] indicates that the "filtrate factor" and the "P-P" factor may not be identical. In addition, Goldberger, *et al.*,[8] have shown that the blacktongue preventative factor is absorbed from yeast by Fuller's earth. However, Koehn and Elvehjem,[9] although unable to cure blacktongue with lactoflavin, found that liver extract, after treatment with alcohol and ether and Fuller's earth, not only contained the chick antidermatitis (filtrate) factor but would cure blacktongue.

On admission to the hospital the pellagrins treated in this study

1 György, P., *Biochem. J.*, 1935, **29**, 741.

2 Elvehjem, C. A., and Koehn, C. J., Jr., *J. Biol. Chem.*, 1935, **108**, 709.

3 Lepkovsky, S., and Jukes, T. H., *J. Biol. Chem.*, 1936, **114**, 109.

4 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

5 Birch, T. W., György, P., and Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

6 Dann, W. J., *J. Nutrition*, 1936, **2**, 451.

7 Jukes, T. H., and Lepkovsky, S., *J. Biol. Chem.*, 1936, **114**, 117.

8 Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *Pub. Health Rep., U. S. P. H. S.*, 1928, **43**, 657.

9 Koehn, C. J., Jr., and Elvehjem, C. A., *J. Nutrition*, 1936, **11**, 67.

were placed on a maize diet similar to the one described by Spies.[10] During the 3 or more days of the control period their condition either remained stationary or became worse.

After remaining on the diet for 3 days, Patient No. 1 received 10 mg. of lactoflavin daily from December 8, 1935, to December 28, 1935. There was a definite improvement in the diarrhea, but the dermatitis and the stomatitis became more severe and dementia developed. The patient then received 6 vials (derived from 600 gm. of liver) of liver extract daily. This medication was followed by a rapid improvement in all symptoms.

Except for slight improvement in diarrhea, all symptoms of Patient No. 2 increased during the first 4 days on the Spies diet. She then received daily for 5 doses 20 mg. of lactoflavin. While on this therapy the diarrhea increased and the caloric intake dropped from a daily average of 1700 calories to 366 calories. The stomatitis and salivation were very severe, and nausea and vomiting and new patches of dermatitis developed. Following the daily administration of 6 vials of liver extract the improvement of all symptoms was very satisfactory.

The dermatitis and the stomatitis of Patient No. 3 became worse during the first 4 days on the diet. At noon of the fourth day the daily administration of 35 cc. (approximately 1 cc. per kilo) of the concentrate "K-50"[4] of the filtrate factor (chick antidermatitis), free from the rat antidermatitis factor and lactoflavin, was begun. The improvement of this patient was quite dramatic. At the end of 10 days of this treatment the mouth was normal, there was no diarrhea, and of the dermatitis there remained only an increase in pigmentation of the skin over the involved parts. Exposure of the face and one hand to direct sunlight for one hour on the tenth day (July 6, 1936) failed to produce a recurrence of any symptoms.

Patient No. 4 had severe polyneuritis in addition to the dermatitis, stomatitis, and severe diarrhea. After 4 days on the Spies diet his general condition was very poor. Because of the predominance of the polyneuritic symptoms, he then received 20 cc. of concentrated vitamin B_1[11] daily intravenously. This product, as prepared by Stuart, is known to contain none of the vitamin B_2 complex.[4] During the following 4 days the general condition improved and the caloric intake increased from 450 to about 1,000 calories per day, but the dermatitis, diarrhea, and stomatitis grew worse. Then 50 cc. of filtrate factor was administered daily. The diarrhea,

10 Spies, T. D., *J. Clin. Invest.*, 1934, **13**, 807.

11 Stuart, E. H., Block, R. J., and Cowgill, G. R., *J. Biol. Chem.*, 1934, **105**, 463.

which had been very severe, rapidly improved, so that by the third day it had completely disappeared. Likewise, the dermatitis and stomatitis rapidly improved.

Pellagrins can be cured while on a maize diet by the administration of a liver filtrate which contains the chick antidermatitis factor but which is free from lactoflavin and rat vitamin B_6.

8925 P

Effect of Cortin upon Renal Excretion and Balance of Electrolytes in the Human Being.*

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Previous attempts to show a positive effect of cortin in normal subjects have met with little success. The present experiments demonstrate that large doses of this hormone produce a prompt effect on the kidney. Sodium, potassium and chloride excretion have been studied in 4 normal subjects and in 2 patients with Addison's disease. All were maintained on a constant diet and liquid intake. Hourly urine specimens were collected during the fasting state with the subject at rest. Cortin was injected intravenously each hour for 4 hours, a total of 80 cat units being injected in each subject. This was approximately 3 times the amount required to maintain a patient with severe Addison's disease for 24 hours. Sodium chloride (0.9%), heated cortin solution and a dilute adrenalin solution were used as control injections. The results are summarized in Table I.

Cortin injections were associated with a marked reduction (average 42%) in the excretion of sodium over a 5-hour period. In patients with Addison's disease similar injections of cortin caused reductions in sodium excretion ranging from 20 to 50% depending on the condition of the patient.

Potassium excretion was increased in the normal subject about 30% for the 5-hour period during which cortin was injected. In the case of one untreated patient there was no increase in potassium excretion during the 5-hour period. With the patients CB[1] and IB[1] the injection of cortin increased the excretion of potassium 66 and 170% respectively during the 5-hour period.

* Aided by a grant from the Rockefeller Foundation.

TABLE I.

Influence of Cortin on Renal Excretion in Normal Human Beings and in Patients with Addison's Disease.

Each subject received 5 cc. of 0.9% NaCl intravenously every hour for 4 hours in experiments "A" and "B". During experiment "B" a total of 80 cat units of cortin were injected with NaCl. Values given below are for total excretion during the 5-hour period after the first injection.

Subject	Urine cc.		Sodium m. eq.		Potassium m. eq.		Chloride m. eq.		Phosphate m. eq.	
	A	B	A	B	A	B	A	B	A	B
A.D.	502	470	32.50	17.11	23.14	30.54	25.48	36.40	40.68	44.18
S.H.	855	836	88.87	42.55	24.63	39.82	60.09	42.35	34.57	29.83
R.M.	830	844	61.57	32.74	36.54	45.01	43.66	29.42	43.57	37.10
G.T.	860	684	49.49	42.10	25.34	26.88	48.63	52.25	38.51	38.42
Patient										
C.B.[1]	373	379	62.20	42.10	6.61	17.96	62.85	47.32		
I.B.[1]	690	381	39.50	30.90	8.49	14.08	35.99	31.96		
I.B.[2]	418	290.5	26.11	13.85	13.84	13.09	31.55	19.37		

[1]Treated with maintenance cortin and NaCl daily.
[2]Untreated.

Some reduction in chloride excretion accompanied the cortin injections in 2 normal subjects and in both patients with Addison's disease.

Electrolyte balance studies were carried out on 8 normal subjects and on 3 patients with severe Addison's disease. The subcutaneous daily injection of from 12 to 30 cat units of cortin produced no effect in the normal subjects.

All 3 patients with Addison's disease showed a marked retention of both sodium (68 to 205 m.eq.) and chloride (66 to 226 m. eq.) following the injection of cortin. Two of these patients had been studied before starting treatment and had been shown to be in a negative balance in regard to both sodium and chloride.

The effect on potassium balance was less marked, although all 3 patients showed a slight negative balance after the injection of cortin.

8926 C

Velocity of Hemocyte Circulation in the Elytron of the Cockroach, *Periplaneta americana* Linn.

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Hemolymph movement in the wings of various insects has been observed by many workers,[1] but no reports on the speed of circulation of the contained hemocytes have come to the notice of the writers. Because of the function that the hemolymph may play in the exchange of respiratory gases through the wing membranes,[2] information which gives direct or indirect indication of hemolymph velocity is of interest. This paper reports the velocity of hemocyte movement in the subcostal cell of the right elytron of the cockroach, *Periplaneta americana* Linn.

The circulating hemocytes were observed by a technic which has been described.[3] In addition a strip of tinfoil was placed under the wing margin in order to reflect more light upward through the elytron. An ocular micrometer was used to measure the length of a portion of a selected hemolymph channel in the subcostal cell area. With the low power of the microscope the course of single hemocytes was followed throughout the length of this channel. The time required for a corpuscle to travel from one end of the measured path (1.7 mm.) to the other was noted by a stop-watch.

A total of 10 determinations with one minute intervals between measurements was made on each of 35 young adult specimens. The results are summarized in Table I.

Considerable variation in hemocyte velocities was encountered in this region of the insect wing. Sex was found not to be responsible for these fluctuations. Age and temperature were controlled factors. All specimens were in approximately the same age group; determinations were made at room temperature. So far it has not been found possible to measure simultaneously heart rate and hemocyte velocity in the wing. In a few cases, however, several series of alternate heart rate and hemocyte velocity determinations were made, but the 2 sets of measurements showed no relationship. Sometimes a fast heart would be associated with high hemocyte velocity;

1 Yeager, J. F., and Hendrickson, G. O., *Ann. Ent. Soc. Am.*, 1934, **27**, 252.

2 Portier, P., *Ve Congres International D'Entomologie*, Part II, 1933, 25.

3 Yeager, J. F., and Hendrickson, G. O., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 858.

TABLE I.
Velocity of Hemocytes in Subcostal Cell of Roach Elytron.

No. of Animal	Maximum Rate (mm./min.)	Minimum Rate (mm./min.)	Aver. Velocity of 10 Determinations (mm./min.)
1	38.5	31.7	33.6
2	63.1	52.9	58.4
3	50.6	43.2	45.2
4	39.0	31.9	34.2
5	61.6	53.3	55.9
6	49.8	41.7	46.9
7	49.7	44.9	46.5
8	25.8	21.9	23.1
9	65.5	64.7	65.2
10	25.9	24.9	25.4
11	19.4	12.9	15.9
12	20.5	18.9	19.7
13	19.4	10.1	16.1
14	49.7	54.3	52.0
15	26.8	29.5	28.5
16	31.7	28.5	29.8
17	29.3	22.2	24.3
18	18.6	12.9	14.5
19	25.4	20.6	22.8
20	42.3	33.7	37.2
21	38.1	30.3	32.1
22	40.9	32.6	36.3
23	41.8	30.4	36.8
24	20.2	15.7	18.7
25	35.1	28.4	30.7
26	20.0	16.1	17.4
27	29.9	24.2	26.3
28	33.1	25.6	29.1
29	34.2	33.6	34.4
30	58.4	55.9	57.1
31	46.9	45.2	46.3
32	22.3	15.8	20.6
33	65.5	63.3	64.4
34	25.9	23.8	25.3
35	34.2	22.8	30.1

Mean of average velocities $\pm\sigma = 34.3 \pm 14.4$ mm./min.
Range of average velocities = 14.5 to 65.2 mm./min.
Highest single rate observed = 65.5 mm./min.
Lowest single rate observed = 10.1 mm./min.

sometimes with a low hemocyte speed. No fixed correlation could be established. If the specimen was made to struggle or was otherwise disturbed, the heart action would become erratic. With the same type of stimulation hemocyte movement would sometimes cease, or become either extremely fast or quite irregular in contrast to the normal steady flow. The width of the channel in which the cells moved was very nearly the same (approximately 0.07 mm.) in all specimens. If the channel became obstructed by bits of tissue or by temporarily quiescent hemocytes the specimen was discarded.

In comparison with blood velocity in man, the range of the average hemocyte speeds (14.5 to 65.2 mm./min.) is considerably

wider than the range of capillary flow rates in man (30 to 54 mm./min.[4]). However, from the standpoint of velocity, the flow in the insect wing channels much more closely approximates the capillary rate of flow in man than the speed of blood in large arteries such as the carotid in which velocities of 18,000 mm./min. (horse) and 15,600 mm./min. (dog) have been recorded.[4]

Summary. The average hemocyte velocity in the subcostal cell of the elytron of the cockroach, *Periplaneta americana* Linn., is 34.3 ± 14.4 mm. per minute. The range of normal average velocities extends from 14.5 to 65.2 mm. per minute. This range approaches the range of 30 to 54 mm. per minute found in the capillaries of man. The highest hemocyte speed observed was 65.5 mm. per minute; the lowest 10.1. No relationship was detected between variations in velocity and sex or the heart rate of the insects used for this study.

8927 C

Relation of Viscosity of Blood to Leucocyte Count, with Particular Reference to Chronic Myelogenous Leucemia.

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Studies of factors which influence the viscosity of the blood have been concerned chiefly with the effect of changes in the total volume of the red blood cells. Nygaard, Wilder and Berkson[1] have recently shown that, within certain limits, the relation between the viscosity of whole blood and the hematocrit value may be well expressed by a linear formula, providing statistical confirmation of similar observations made by Allbutt,[2] Austrian[3] and Bircher.[4] The probable importance of the white blood cells in contributing to significant changes in the viscosity of the blood has been considered by a number of workers. Bircher[4] stated that the white blood cells did not influence the viscosity of normal blood because of their

4 Howell, W. H., Textbook of Physiology, 11 edition, 1930, 493.

1 Nygaard, K. K., Wilder, M., and Berkson, J., *Am. J. Physiol.*, 1935, **114**, 128.

2 Allbutt, C., *Quart. J. Med.*, 1910, **4**, 350.

3 Austrian, C. R., *Bul. Johns Hopkins Hosp.*, 1911, **22**, 9.

4 Bircher, M. E., *J. Lab. and Clin. Med.*, 1921, **7**, 134.

small number but pointed out that if sufficiently increased they might have a marked influence. High, normal and low values for the blood viscosity in leucemia have been recorded by several observers.[2-7] This communication reports the results of a quantitative study of the relation between the white blood cell count and volume and the viscosity of whole blood, with particular reference to the leucocytosis of chronic myelogenous leucemia.

Simultaneous observations of the viscosity of whole blood, the hematocrit value and red and white blood cell counts were made in patients with chronic myelogenous leucemia at various blood levels. The viscosity was measured in relation to distilled water at room temperature by means of the Hess viscosimeter.[4] Hematocrit values

TABLE I.

Blood Counts, Hematocrit Values and Blood Viscosity in Chronic Myelogenous Leucemia.

Subject	Date	R.B.C. Millions per cu. mm.	W.B.C. per cu. mm.	Hematocrit Volumes % R.B.C.	Hematocrit Volumes % W.B.C.	Viscosity	Circulation Time sec.
E.G.	4-15	3.17	463,000	25.5	29.0	9.4	
	4-20	3.41	379,000	28.0	21.0	7.2	
	4-25	4.02	103,000	33.5	8.5	5.1	
	4-30	3.89	58,000	36.0	3.0	4.6	
	6-19	4.34	358,000	33.0	20.0	7.8	
	6-24	3.87	246,000	32.5	17.0	7.0	
	8-25	3.60	306,000	28.0	24.0	7.6	
	8-31	3.21	552,000	26.0	29.0	9.8	22
	9-8	3.79	47,000	32.5	3.5	3.8	14
J.N.	513	3.72	383,000	25.5	18.5	7.0	19
	5-19	3.17	338,000	21.0	17.0	5.5	
	5-22	2.71	252,000	23.0	14.0	4.8	
	5-28	2.92	58,000	26.0	3.0	3.4	
	6-1	2.95	14,900	28.0	1.0	3.6	13
M.T.	5-12	4.55	87,000	38.0	8.0	6.4	
	5-26	4.16	142,000	31.0	12.0	5.9	
	8-23	4.28	97,000	34.0	8.0	5.6	
F.S.	4-28	4.71	45,000	33.0	2.5	4.0	
	5-26	4.09	35,300	32.0	3.0	3.8	
	6-23	4.38	58,000	33.0	5.0	3.9	
F.R.	5-8	4.03	54,000	32.0	3.0	4.2	
	9-4	4.77	5,850	37.0	0.5	4.8	
A.S.	4-30	4.60	73,000	44.5	5.0	5.8	
M.F.	9-9	3.29	347,000	24.0	12.5	6.8	26

[3] Determann, Dr., *Z. f. klin. Med.*, 1906, **59**, 282.

[6] Retky, H., *Z. f. Heilkunde*, 1907, **28**, 106.

[7] Naegeli, O., Blutkrankheiten und Blutdiagnostik, Julius Springer, Berlin, 1931, 41.

were determined by the Wintrobe method,[8] using the anticoagulant recommended by Heller and Paul.[9] The red and white blood cell counts were determined in the usual manner. Table I shows the results of 24 such determinations. Examination of the data shows, as might be expected, that the viscosity of whole blood in myelogenous leucemia is determined not only by the level of the white blood cell count and volume but also by the relative volume of the red blood cells.

In order to study the relation of the white blood cell hematocrit to the viscosity more directly, a number of observations were made of suspensions of white blood cells in plasma. By repeated sedimentation of a large sample of oxalated whole blood obtained from patient E. G., a suspension of leucocytes in plasma, free of erythrocytes, was obtained. This suspension contained 600,000 white blood cells per cu. mm., 34% of white blood cells by volume and a viscosity of 4.4. A number of dilutions of this suspension were made by the addition of appropriate amounts of plasma (viscosity, 1.7) obtained from the same patient. Hematocrit and viscosity determinations made in each of these dilutions are shown in Fig. 1. Within the range covered by this experiment there is apparently a linear relation between the relative volume of leucocytes suspended in plasma and the viscosity of such a suspension.

As a corollary to the above experiment, the relation between the total leucocyte count and the white blood cell hematocrit of such suspensions and of whole blood was investigated (Fig. 1). The relation is apparently linear and is similar to that recorded by Wintrobe[8] in a series of determinations made in blood samples in which the predominating leucocytes were of the myeloid series. It should be noted that the observations recorded in Fig. 1 were made in bloods obtained from patients with chronic myelogenous leucemia, in which cells of the myeloid series constituted from 85 to 99% of the total leucocytes. The average size of the white blood cells, calculated from data shown in Fig. 1 was 628 cubic microns, with extremes of 417 and 920 cubic microns. The relations depicted would not be applicable to blood samples containing significantly larger numbers of lymphocytes, the volume of which has been found to be between 170 and 300 cubic microns.[8] Because of the limited number of observations and the nature of the data (varying proportions of immature and mature granulocytes in the samples examined) the determination of statistical constants was not considered feasible.

[8] Wintrobe, M. M., *Am. J. Med. Sci.*, 1933, **185**, 58.

[9] Heller, V. G., and Paul, H., *J. Lab. and Clin. Med.*, 1934, **19**, 777.

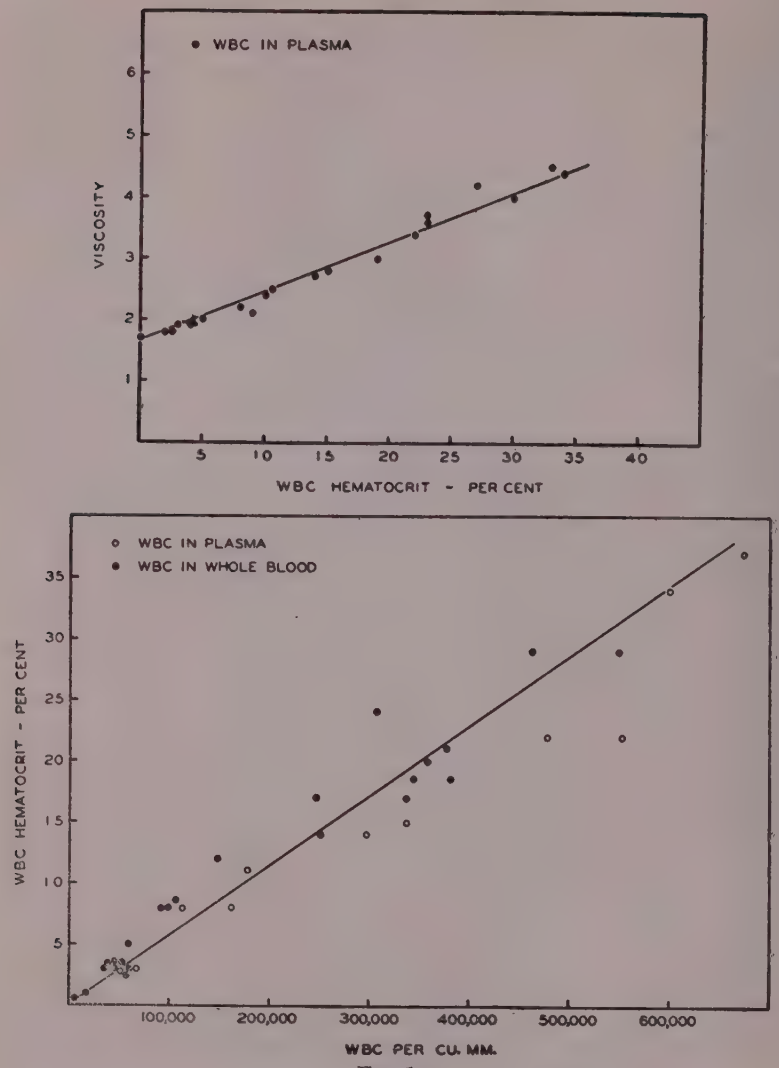

FIG. 1.

The relation of the white blood cell hematocrit to viscosity and to the total white blood cell count.

From the data presented it is apparent that the leucocytes had little effect on the viscosity of whole blood unless the total white blood cell count was in excess of 50,000 per cu. mm. and the white blood cell hematocrit exceeded 3 volumes %. With more marked elevations in the leucocyte count, significant increases in the blood viscosity were observed, the extent of which was dependent to some

degree on the relative volume of the erythrocytes. Attempts to demonstrate a constant relationship between the total hematocrit and blood viscosity were unsuccessful because of a disproportionate increase in the latter in the higher hematocrit ranges. Bircher[4] states that the influence of the various factors which determine the blood viscosity is not merely a summation but is rapidly intensified with increases in concentration. Our observations support this contention. In this regard, it is of interest that Nygaard, Wilder and Berkson[1] observed that for high values of hematocrit observed in patients who were suffering from polycythemia, the viscosity was higher than the values predicted from their formula.

In view of the very high values observed in several instances (Table I), it seems not improbable that the increased blood viscosity accompanying very high leucocyte counts in myelogenous leucemia may account for some of the symptoms observed in this disease. In chronic lymphatic leucemia normal values for the blood viscosity[7] have been observed in the presence of leucocyte counts in excess of 500,000, presumably owing to the small relative volume of the lymphocytes. Lengsfeld[10] called attention to a certain parallelism between polycythemia and myelogenous leucemia with high leucocyte count and suggested that the cerebral symptoms observed in his patient may have been due to a probable great increase in viscosity accompanying a white blood cell count of 800,000 per cu. mm. Rotenberg[11] has recently expressed the opinion that increased viscosity of the blood may play a part in the pathogenesis of priapism occurring in leucemia and other diseases.

Observations of the arm to tongue circulation time (Decholin method) were made in 3 patients with marked elevation of the leucocyte count, hematocrit and blood viscosity (Table I). Patient E. G. repeatedly complained of dizziness, roaring sensations in the ears, mental dullness and faintness during periods of marked leucocytosis. These symptoms disappeared when the white blood cell count was reduced by appropriate treatment. A circulation time of 22 seconds, which accompanied a blood viscosity of 9.4, was reduced to a normal value when the total white blood cell count, hematocrit and viscosity were lowered by means of roentgen therapy. Similar observations were recorded in the case of J. N., who had recently experienced symptoms and signs characteristic of splenic infarction. Patient M. F., who was seen through the courtesy of Dr. F. K. Holzwarth, presented the typical picture of chronic myelo-

[10] Lengsfeld, W., *Jahrb. f. Kinderheilkunde*, 1929, **126**, 289.
[11] Rotenberg, M. I., *J. D'Urologie*, 1935, **39**, 508.

genous leucemia, complicated by transverse myelitis of the thoracic spinal cord. Marked prolongation of the circulation time and increased blood viscosity accompanied a high leucocyte count and white blood cell hematocrit.

Summary. 1. Observations of the blood viscosity, hematocrit, blood counts and circulation time were made in a group of patients suffering from chronic myelogenous leucemia. 2. The relation between viscosity of the blood and the total number and volume of the white blood cells was determined. 3. The high leucocyte counts observed in chronic myelogenous leucemia are frequently responsible for marked increase in blood viscosity and prolongation of the circulation time. The probable relation of these changes to the symptomatology of the disease is briefly discussed.

8928 C

Seasonal Variation in Susceptibility of Animals to Tetanus Toxin.

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During the course of certain investigative work with tetanus toxin,* it was noted that there appeared to be a consistent variation in the susceptibility of animals to the same lot of toxin. Judging from the time of onset and the severity of the symptoms in acute and subacute poisoning in the rabbit and guinea pig, there was found an increased susceptibility during the summer months and decreased susceptibility during the winter months. Since this observation was rather incidental, it was decided to determine the actual minimal lethal dose of the toxin at various periods during the year.

For these toxicity studies, guinea pigs were used in all experiments. The toxin was kept in an icebox in small vials sealed in carbon dioxide. Approximately 100 guinea pigs were used. The work was carried on over a 2 year period.

The minimal lethal dose as determined during October and February and a year later in November was found to be from 0.004 to 0.005 mg. per kilo of body weight. This amount will kill approximately 8 out of 10 animals. All animals die with 0.006 mg. per kilo.

* The tetanus toxin for this work was kindly furnished by Dr. McCoy of the National Institute of Health, Washington, D. C.

The minimal lethal dose during the months of June a year apart was from 0.002 to 0.003 mg. per kilo. This amount again killed approximately 8 out of 10 animals and all died with 0.004 mg. per kilo.

If the toxin be thought to change spontaneously on long standing, it of necessity should become either less or more potent. Since identical figures were obtained during the same seasons a year apart, *i. e.*, greater toxicity both summers and lesser toxicity both winters, we are forced to conclude that the differences observed reside in the conditional state of the animals and not to changes in the toxin.

It seems to us, therefore, that there is a sufficient variation in the seasonal susceptibility of guinea pigs to tetanus toxin to justify special care in its standardization.

By contrast to the above data on tetanus toxin, it may be noted that diphtheria toxin appears to be more toxic to pigs in winter than in summer.[1] The two toxins, however, are obviously quite different in character and in mode of action, hence further knowledge is required in order to correlate the differences in the two toxins with respect to seasonal variations of susceptibility of guinea pigs and rabbits.

8929 P

Epinephrine Secretion in Animals with Experimental Diabetes.

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Rogoff and Ferrill[1] have shown that the development and course of diabetes following total extirpation of the pancreas, in dogs, is not modified by reduction or suppression of epinephrine secretion, from the adrenal glands. Further, they observed that in depancreatized dogs, not subjected to operations for interference with epinephrine secretion, a marked reduction in the epinephrine output may occur after various periods, under treatment with insulin on a constant diet.

We are investigating the probable causes for this disturbance of

1 *British System of Bacteriology*, 1931, **6**, 108.

* Aided by the G. N. Stewart Memorial Fund and a grant from Mr. Max Manischewitz.

We are grateful to Eli Lilly & Company for generous supplies of insulin.

1 Rogoff and Ferrill, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 100.

the epinephrine secretion from the adrenals and have attempted to determine whether the following factors play a rôle: *a*, the diabetic state, *b*, a possible influence of insulin, *c*, integrity of the splanchnic innervation. This report is a summary of the results obtained by us in experiments on 39 dogs.

Sixteen animals (Group 1) were totally pancreatectomized and kept on a diet consisting of 500 gm. boiled beef lung, 100 gm. cane sugar and 75 gm. fresh raw beef pancreas daily. Insulin was administered in doses necessary to maintain the daily excretion of sugar in the urine below about 5 gm. and below about 1%. At different periods of observation, ranging from 11 to 77 days following pancreatectomy, experiments were terminated and the animals sacrificed for determination of the epinephrine output from the adrenal glands by the method of Stewart and Rogoff.

Thirteen dogs (Group 2) were totally pancreatectomized and kept on a diet of 500 gm. boiled beef lung and 75 gm. fresh raw pancreas daily. Sugar was not added, nor were they treated with insulin. The animals were sacrificed for determination of epinephrine output from the adrenals when they showed evidence of severe diabetes, 4 to 23 days after pancreatectomy.

Ten normal, unoperated dogs were kept on a diet of 500 gm. boiled beef lung and 100 gm. sugar daily. Five of these animals (Group 3) were injected with insulin in doses comparable with those employed in depancreatized animals of Group 1. The other 5 animals (Group 4) received no insulin. Epinephrine output from the adrenals was determined after 22 to 64 days of observation in Group 3 and after 21 to 55 days in Group 4.

Under ordinary experimental conditions, the average epinephrine output in dogs (and cats) is approximately 0.0002 mg. per kg. body weight, as determined by Stewart and Rogoff.[2] In about 85% of a large series of determinations the limits were 0.0001 to 0.0003 mg. per kg. These figures may be used for comparison with the figures for epinephrine output in the present series of experiments, since the determinations were made with the same method.

Of the 16 animals in Group 1, the epinephrine output was within the ordinary range for normal animals in 4 experiments, Six showed an output corresponding to about 1/3 to 1/2 of the lower level of the normal range, or about 1/6 to 1/4 of the average. The epinephrine output in the other 6 animals ranged from 1/10 to 1/75 of the average for normal dogs. In Group 2 (13 dogs), 10 showed a normal epinephrine output, in one the output was 1/5 and in 2 approximately 1/20 of the normal average. Of the 5 animals in

[2] Stewart and Rogoff, *Am. J. Physiol.*, 1923, **66**, 235.

Group 3, one showed a normal output and the others ranged from 1/20 to 1/100 of the average epinephrine output for normal dogs. In Group 4 (5 dogs) a normal output was found in 3 animals, about 1/8 of the normal average in one and 1/50 in the other.

It appeared, at first, that insulin might be responsible for the low epinephrine output, since most of the treated depancreatized animals showed a marked reduction. However, in 3 of the 13 untreated depancreatized dogs there was a definitely reduced epinephrine output. The animals in this group did not live nearly as long as the treated animals, which may explain the smaller number of instances of reduction in output in the untreated dogs. On the other hand, a similar difference is seen in the case of unoperated dogs on a diet rich in sugar, between Groups 3 (receiving insulin) and 4 (untreated). Of course, it is possible that this difference might be less striking in larger series of experiments. Nevertheless, whatever the reason may be for the greater number of instances of reduced epinephrine output in the insulin-treated groups, it is evident that this reduction can occur without the action of insulin. That the liver may play a rôle and other probable factors remain for further investigation.

The evidence indicates that the diabetic state of the animal is primarily responsible for the reduction or suppression of epinephrine secretion from the adrenals. We have found that electrical stimulation of the splanchnic nerve is capable of increasing the epinephrine output up to or above the normal level, in those animals that show a marked reduction in output.

8930 C

Electrocardiographic Changes in Rats Deficient in Vitamin B_1.

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Vitamin B_1 deficiency in rats and pigeons has been found to be associated with bradycardia (Birch and Harris[1]; Drury, Harris and Maudsley[2]; Carter and Drury[3]; and Méhes and Péter[4, 5]). Changes

1 Birch, T. W., and Harris, L. J., *Biochem. J.*, 1934, **28**, 602.

2 Drury, A. N., Harris, L. J., and Maudsley, C., *Biochem. J.*, 1930, **24**, 1632.

3 Carter, C. W., and Drury, A. N., *J. Physiol.*, 1929, **68**, i (Proceedings).

4 Méhes, J., *Arch. f. Exp. Path. u. Pharm.*, 1934, **176**, 141.

5 Méhes, J., and Péter, F., *Arch. f. Exp. Path. u. Pharm.*, 1934, **176**, 226.

in the complexes of the electrocardiograms have not been described. As far as one can ascertain, the electrocardiograms obtained in rats deficient in vitamin B_1 were not standardized.

Because of the discrepancy between electrocardiographic findings in deficiency states attributed to vitamin B in man (beriberi, pellagra, polyneuritis)[6,9] and in animals, a study was undertaken on the effect of vitamin B_1 deficiency on the heart of the rat, as indicated by the electrocardiogram. Nine rats were placed on diets deficient in B_1, consisting of Wesson[10] salt mixture 3.5%, starch 55%, butter fat 8.5%, casein 18% and autoclaved bakers' yeast 15%. The casein was washed with alcohol by a method similar to that of Chase and Sherman.[11] The yeast was autoclaved for one hour at 20 pounds after the addition of 0.1 normal sodium hydroxid. Standardized electrocardiograms using skin copper leads were obtained. During a period of 7 weeks only moderate loss of weight developed. This, together with the absence of bradycardia and of nervous manifestations, indicated the existence of but partial deficiency. The yeast was therefore autoclaved for 6 hours at 15 pounds at a pH of 8 to 9. After 3 weeks on this modified diet the animals exhibited marked loss of weight, neurological manifestations, bradycardia and changes in the electrical complexes of the cardiogram. The heart rate gradually fell from a normal level of from 564 to 666 per minute (average 581) to from 354 to 134 (average 286). In 5 of the rats T wave changes of high origin were observed. Increase in the height of the T wave developed in 2 animals. Definite inversion of the T wave occurred in one, and questionable inversion in another animal. In one of the deficient animals there were no changes in the complexes. The alterations in the electrocardiographic complexes bore no direct relation to the degree of slowing of the heart.

Subcutaneous administration of from 5 to 25γ of crystalline vitamin B_1 (Merck) abolished both the bradycardia and the changes in electrocardiographic complexes when the rate was 300 or over, but usually failed to save the animals when the rate was below this level. Elevation of the heart rate and disappearance of the abnormal complexes occurred as early as within 4 hours. In animals in

6 Scott, L. C., and Herrmann, G. R., *J. A. M. A.*, 1928, **90**, 2083.

7 Keefer, C. S., *Arch. Int. Med.*, 1930, **45**, 1.

8 Feil, H., *Am. Heart. J.*, 1936, **11**, 173.

9 Weiss, Soma, and Wilkins, R. W., *Tr. Assn. Am. Phys.*, 1936, **51** (in press).

10 Wesson, L. G., *Science*, 1932, **75**, 339.

11 Chase, E. F., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 3506.

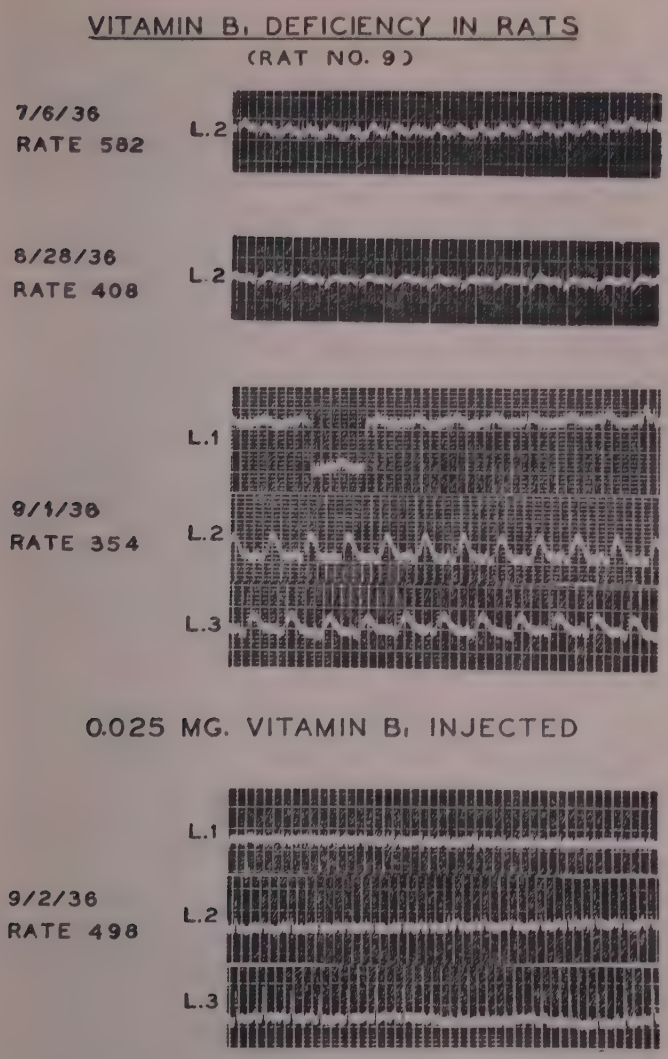

FIG. 1.

The effect of diet deficient in vitamin B_1 on the electrocardiogram of the rat. Note progressive slowing of the cardiac rate and changes in the S T complexes. Subcutaneous injection of crystalline B_1 abolished the changes in the electrocardiographic complexes within 12 hours.

which the deficiency was abolished with crystalline B_1 and was repeatedly induced again, the changes in the electrocardiographic complexes during the subsequent deficiency state were not always identical. The fact that the changes in the electrocardiographic

complexes could be abolished with crystalline vitamin B_1 in animals kept in a fasting state indicates that the cardiac changes are directly related to B_1 deficiency rather than to malnutrition. The latter factor may nevertheless play a secondary rôle. Fig. 1 represents the result of an experiment on the effect of vitamin B_1 deficiency and of the administration of crystalline B_1.

Two rats, used as controls, were kept on an identical diet with the single exception that the yeast was not autoclaved. These animals gained weight and exhibited no cardiac slowing, changes in the electrocardiographic complexes nor nervous manifestations. Further experiments are in progress to elicit the relationship between the cardiac changes here described and vitamin B_1 deficiency.

The results of the experiments here reported and the character of the electrocardiographic changes described are in harmony with the electrocardiographic changes observed in human deficiency states (pellagra, polyneuritis, beriberi). The essential difference is that in man tachycardia rather than bradycardia is present in deficiency states attributed to vitamin B. Transient bradycardia has been observed during recovery from severe "beriberi heart".[9]

8931 C

A Comparative Assay of Black Widow Anti-Sera.

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This paper reports the results of a comparative assay of the recently perfected super-immune serum from sheep* and a sample of convalescent human serum supplied through the courtesy of Dr. Emil Bogen of Olive View, California. Some success has been reported in the treatment of arachnidism (of which there were 615 reported cases with 38 deaths in 1935) with human serum and it seemed desirable to compare its potency with that of a carefully assayed super-immune animal serum.

The assay was carried out as follows: A solution of venom was prepared by dissecting the venom-glands from spiders, macerating and dissolving in saline. The average lethal dose (A.L.D.), that is, the dose required to kill 50% of the test animals, was determined. Varying amounts of the sera were added to solutions of the venom,

* Anti-Black Widow Spider Serum—Squibbs.

allowed to stand in the refrigerator over night and injected. The results are shown in Table I.

TABLE I.

No. of Rats	Material Injected per Rat	No. Killed	% Killed
	Sheep Super-Immune Serum		
10	10 A.L.D. plus 0.1 cc. serum	7	70
10	10 " " 0.2 " "	5	50
10	10 " " 0.4 " "	0	0
	Human Convalescent Serum		
7	1 A.L.D. plus 0.25 cc. serum	4	57
6	1 " " 0.5 " "	4	66
4	2 " " 1.0 " "	4	100
	Normal Sheep Serum		
10	2 A.L.D. plus 1.0 cc. serum	10	100

Conclusions. The results indicate a much higher potency for the sheep serum, one cc. of this serum completely neutralizes 25 average lethal doses of the venom whereas one cc. of the human convalescent serum shows no neutralizing power whatever against 2 average lethal doses of venom.

8932 C

Physical Chemistry of Lipoids. IV. Influence of Narcotics on the Salt-binding Capacity of Lecithin.

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Direct reactions between lipoids and narcotics were missed in previous studies of viscosimetry[1] and interferometry.[2] In view of the biological importance of lipoids, one must therefore assume that narcotics influence reactions of lipoids with other substances. Nervous excitation is explained by Nernst[3] by changes of ion concentrations occurring at the surface of membranes. Experimental proof of the important rôle of lipoids in artificial polarizable membranes has recently been given.[4] The importance of lipoids in narcosis[5] has been widely assumed. Nevertheless, no data are available that

1 Handovsky, H., and Wagner, R., *Biochem. Z.*, 1911, **31**, 32.
2 Spiegel-Adolf, M., *Biochem. J.*, 1932, **26**, 2183.
3 Nernst, W., *Pflüger's Arch.*, 1908, **122**, 275; **123**, 454.
4 Spiegel-Adolf, M., *J. Biol. Chem.*, 1936, **114**, xcix.
5 Henderson, V. E., *Physiol. Rev.*, 1930, **10**, 171.

connect the changes of ion concentration on the cell surface with the lipoids. Former experiments[6] on the salt-binding capacity of lecithin were therefore continued, and the influence of narcotics was examined. The lecithin sols made from egg-lecithin (extra pure Merck, Germany) were either emulsions or were prepared by the method of Keeser[7] (pouring small amounts of boiling alcoholic lecithin solutions into boiling distilled water and boiling the alcohol away). In view of former observations[2] only fresh samples of lecithin sol were used.

Since higher concentrations of lecithin were to be used, conductivity measurements were made instead of the formerly used interferometric determinations. The conductivity (K) was measured in the aqueous lecithin solutions (a), in the salt solutions (b), and in an aqueous solution (c) containing lecithin and salt in the same concentrations as in a and b respectively. The difference (KD) between the calculated values (a + b) and the observed value (c) is used as a measure for the amount of ions bound or adsorbed to lecithin. A similar method has been used by Spiegel-Adolf[8] on globulins. In all experiments it could be shown that the electric conductivity of KCl is decreased by the presence of lecithin. In analogy with the findings in proteins, this fact can be explained by a binding or inactivation of salt-ions by the colloidal lecithin particle.

Quantitatively the salt-binding capacity of lecithin sol depends upon several factors.

TABLE I.*
Influence of Ageing.

Time after mixture	5 hr.	24 hr.	48 hr.	72 hr.
KD $.10^{-3}$	1.22	1.62	1.95	2.12

* All figures in Tables I-IV refer to 1% lecithin sol. 0.05 N KCl (except Table II) and were measured at 30° C.

1. The results summarized in Table I indicate that the salt-binding capacity of lecithin-sol increases with the time the mixture is allowed to stand. It could be ascertained by special experiments that these changes do not depend upon the ageing of the lecithin sol but upon the time the salt is in contact with the colloid.

2. The amount of salt bound to lecithin sol depends upon the concentration of both salt and lecithin. If the concentration of lecithin sol is kept constant, and the amount of KCl is varied, then the

6 Spiegel-Adolf, M., *Biochem. J.*, 1935, **29**, 2913.
7 Keeser, E., *Biochem. Z.*, 1924, **154**, 321.
8 Spiegel-Adolf, M., *Kolloidchem. Beih.*, 1923, **18**, 275.

graphical presentation suggests the existence of a salt binding maximum in a surplus of salt.

3. The amount of salt bound to lecithin sol depends upon the degree of dispersion of the lecithin sol. Bungenberg, Verberg and Westerkamp[9] have shown recently that even small amounts of contamination prevent the making of a translucent lecithin sol. When a different preparation (Merck, Rahway. 90% pure) was used, the lecithin sol became opaque and milky and the salt-binding capacity of the lecithin sol dropped to about 1/10 of the original value.

In order to elucidate the type of salt fixation to lecithin sol, 2 different series of experiments were undertaken. 1. In aqueous solutions lecithin is negatively charged.[10] Electrophoresis experiments were made using the apparatus of Landsteiner and Pauli.[11] The KCl concentration was varied. Up to 0.1 KCl, no change of the anodic movement of the lecithin sol could be detected. 2. In systematic investigations on globulins[8] it could be shown that globulin calls forth different decreases in conductivity in isonormal solutions of KCl, NaCl and LiCl. This has been explained by the different velocities of the cations and was regarded as a proof that part of the cations had been bound to the globulin. Similar experiments made with lecithin sol gave analogous results. (Table II.)

TABLE II.
Influence of Cation-Velocity.

Salt	KCl	NaCl	LiCl
KD .10^{-3}	1.62	1.04	0.96

The results of both the electrophoresis and the conductivity measurements seem to indicate that both salt ions are fixed by the lecithin, and that the new compound shows little if any ionisation. Under this assumption, 1% lecithin allowed to stand for 24 hours in contact with 0.05 N KCl binds approximately 18% of the salt.

In another series of experiments, the influence of some narcotics on the salt-binding capacity of lecithin was investigated. In every case all the samples (lecithin-sol, salt solution, lecithin-salt mixture) were treated in the same way in order to exclude possible interferences of changes of the dielectric constants. Some of the results are summarized in Table III.

[9] Bungenberg de Jong, H. G., Verberg, G., and Westerkamp, R. F., *Kolloid Z.*, 1935, **71**, 184.

[10] Thierfelder, H., and Klenk, E., Die Chemie der Cerebroside und Phosphatide, Berlin, J. Springer, 1930.

[11] Landsteiner, K., and Pauli, W., *Verh. d. Kongr. f. Inn. Med.*, 1908, **25**, 571.

TABLE III.
Influence of Narcotics.

	Ethyl Alcohol	Ether	Chloroform	Chloral hydrate
Lecithin Salt Narcotic, KD_0 E.10⁻⁴	1.11	1.10	1.11	1.08
Control without Narcotic, KD_0.10⁻⁴	1.05	2.68	2.12	2.18
KD_0 KD_0.10⁻⁴	3.4	15.3	10.1	2.0

The figures show that the presence of ethyl alcohol, ether, chloroform, and chloralhydrate decreases the salt binding capacity of lecithin. Since these substances are chemically different, no attempt was made to compare their effectiveness on the salt binding capacity of lecithin in a quantitative way. For this purpose homologous alcohols were used, since these alcohols have been studied, especially in order to correlate chemical and biological behavior.[13] The results are summarized in Table IV.

TABLE IV.
Influence of Homologous Alcohols.

	KD	Dielectric[12] constants Aqua — 80	Partition coeff. 5) Oil: water	Sorption[13] Picric acid to charcoal	Narcotic effect 5)
Methyl Alcohol	95 x 10⁻⁴	32	50	98	.57
Ethyl "	58 "	26	1.30	91	.39
N Propyl "	53 "	22	1.18	75	.31
I Butyl "	10 "	19	6.1	64	.045
N Butyl "	2 "	—	8.12	—	.038
I Amyl "	0 "	16	8.12	—	.023

[12] Wintersteln, H., Die Narkose, 1926, Springer, Berlin.

[13] Thomas, A. W., Colloid Chemistry, 1934, McGraw-Hill, New York and London.

The results show that the capacity of alcohols to decrease the salt binding capacity of lecithin becomes more marked as the length of the chain increases. In presence of amyl alcohol the salt binding capacity has practically disappeared. At the same time the opacity of the lecithin sol increases, as well as its sensitiveness to salt precipitation. A similar behavior has been observed by Freundlich and Kona,[14] when colloidal ferric hydroxyde was treated with narcotics.

Several possible explanations are to be considered: 1. the dielectric constants of the homologous alcohols decrease with increasing chain length. It has been mentioned above that the experimental method used excluded an influence of changes in the dielectric constants upon the salt conductivity. Nevertheless, in presence of a

[14] Freundlich, H., and Kona, P., Sweden 2., 1917, 181, 87.

lipoid, such changes could account for a loss of conductivity, as long as the narcotic is more soluble in water. But this explanation fails with the higher alcohols in which the lipoid solubility prevails. 2. It seems more probable that the different alcohols differ in effect on the sorption capacity of colloidal lecithin. Freundlich and Rona[14] have explained the sensitizing action of narcotics upon ferric sol through sorption of the narcotics to the surface of the colloid. Heymann and Boye[15] working on the same series of alcohols have shown a very similar phenomenon to the one observed on lecithin. In their experiments, the sorption capacity of charcoal for various acids decreased with increasing chain length and molecular polarization of the added alcohols. 3. The relation between salt-binding capacity and degree of dispersion in the lecithin sols have been mentioned above. Therefore, in the case of lecithin, the influence of these alcohols upon the dispersion of the sols should in itself lower the salt binding capacity of lecithin. Chloralhydrate which has less effect upon the salt binding capacity of lecithin sol has been found to increase the dispersion of lecithin sol.[7] If it is warranted to assume that egg lecithin behaves physicochemically like human lecithin, then the diminished ability of the lipoids in the cellular surface films to fix salt ions should diminish the reactivity of the cell upon stimuli. Changes in ion concentrations must reach a higher threshold in order to act upon the lipoids of the cell surface or of its interior. In this connection, it seems of interest that there is a certain parallel between the power of homologous alcohols to lower the salt binding capacity of lecithin and their narcotic effect.

8933 P

Localization of Pain Following Faradic Stimulation of the Common Bile Duct.

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Previous studies[1] have demonstrated the inability to reproduce pain referred to the back by mechanical distention of the gallbladder or common duct in conscious patients. Pain, characteristic of the

[15] Haymann, E., and Boye, E., *Z. f. phys. Chem.*, 1930, 150A, 219.

[1] Zollinger, Robert, Proc. Soc. Exp. Biol. and Med., 1933, 30, 1260.

referred pain described preoperatively, was reproduced by faradic stimulation of 8 patients in whom an electrode was incorporated in the common duct catheter at operation. These patients had chronic cholecystitis and cholelithiasis with well defined indications for choledochostomy.

Method. A platinum wire was incorporated in a small soft rubber common duct catheter adjusted to present an uninsulated area, one-fourth of an inch beyond the tip of the catheter. Following exploration of the common duct through a longitudinal incision distal to the entrance of the cystic duct, the catheter electrode was inserted upward for a distance of about one inch. The end of the electrode was usually at the bifurcation of the hepatic ducts, some distance from the pancreas and duodenum. The position of the electrode, as demonstrated by roentgenograms in the majority of patients, was at the level between the eleventh and twelfth rib, approximately 1½ inches to the right and 2½ inches anterior to the vertebral bodies. At intervals during the first 12 postoperative days a stimulus of faradic current was applied to the catheter electrode from a Harvard inductorium using 3 volts on the primary circuit.

Results. The radiation of the pain differed on various days during the convalescent period. The majority of the patients complained of distress in the epigastrium or right upper quadrant on stimulation similar to their preoperative pain. Four patients also complained of pain in the back, either in the midline at the level of the electrode or with as frequent radiation to the left as to the right. Three patients at some time referred pain to the right side of the abdomen, about 3 inches to the right of the umbilicus. One patient experienced pain in the left epigastrium in the neigborhood of the tip of the eighth rib.

In 3 patients barium was given by mouth and fluoroscopy carried out during faradic stimulation. These patients complained of epigastric distress following stimulation, but in only one did peristaltic waves at the pylorus accompany the stimulation and discomfort.

Conclusions. The sites of pain following faradic stimulation of the common duct are not restricted to the anterior abdominal wall as found following electrical excitation of the stomach and duodenum.[2] The location of the pain coincided more closely with the level of the electrode in relation to the cerebro-spinal segment involved. Pain was referred to the back in those who described such radiation preoperatively. The epigastric distress at times seemed to coincide with peristaltic contractions of the pylorus.

[2] Boyden, Edward A., and Rigler, Leo G., *J. Clin. Invest.*, 1934, **13**, 833.

8934 P

Effect of Progestin and Progesterone on Ovulation in the Rabbit.

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Although the post partum rabbit (oestrus) uniformly ovulates after coitus, the pregnant or pseudopregnant rabbit does not ovulate after coitus. This failure of post-coital ovulation in the pregnant or pseudopregnant rabbit, as well as the absence of spontaneous ovulation during pregnancy in other species, has been attributed to some inhibitory influence of the corpus luteum. It is obvious that this inhibition could be effected either by rendering the ovarian follicles refractory to the normal concentrations of the gonadotropic hormone, or by interfering with the normal supply of this hormone to the ovarian follicles.

To determine the mechanism of this inhibition, post partum rabbits were injected daily with varying doses of progestin* or progesterone† for 5 days. At the end of the fifth day an attempt was made to mate the treated animals. Those animals which refused the male were immediately injected with one minimal ovulating dose of pregnancy urine extract‡ which had just been closely assayed by the rabbit method.[1, 2] Laparotomy was performed 18-24 hours later to determine whether or not ovulation had occurred.

Of the 24 females which had been injected with corpus luteum preparations, 9 accepted the male. In not a single instance did coitus provoke ovulation. The remaining 15 females were injected with the P. U. extract, and this was followed by ovulation in 10 animals. From these results it is clear that post-coital ovulation in the oestrous rabbit is prevented by the daily injection of corpus

* Progestin—A relatively pure extract made from sow corpora lutea by the method of Allen and Meyer (1933).

† Progesterone—Crystalline hormone, synthesized from stigmasterol. A portion of this material was obtained through the courtesy of Dr. Erwin Schwenk of the Schering Corporation.

‡ Pregnancy urine extract—Antuitrin S, a relatively stable benzoic acid extract of pregnancy urine, was obtained through the courtesy of Dr. Oliver Kamm, of the Parke Davis Co.

1 Friedman, Maurice H., *J. Pharm. and Exp. Therap.*, 1932, **45**, 7.

2 Rowe, L. W., Simond, A., and Nelson, W. O., *J. Am. Pharm. Assn.*, 1934, **23**, 882.

luteum preparations in amounts equal to, or greater than, the quantity necessary to sustain pregnancy in the castrate rabbit.[8] It is equally clear that this inhibition of ovulation is not effected by altering the sensitivity of the ovarian follicle as measured by the response to P.U. If we may accept our injections of crystalline progesterone as a satisfactory hormonal substitution for the corpus luteum of pregnancy or pseudopregnancy, we might then conclude that the corpus luteum hormone suppresses ovulation not by any direct action on the ovarian follicles, but by some interference at a site more central in the chain of the ovulation-provoking mechanism.

8935 C

Periphytic Habits of Some Marine Bacteria.

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In studying the factors which influence the increased bacterial activity[1] during the storage of samples of sea water collected for bacteriological analysis, ZoBell and Anderson[2] noted that multiplication occurs more rapidly in small volumes than in large volumes of the water. Similar observations were made on fresh water by Whipple[3] who reported that after 24 hours' storage there were 300 bacteria per cc. in a gallon, 7,020 per cc. in a pint and 41,400 per cc. in 2 ounces of water which initially contained 77 bacteria per cc. Whipple attributed the difference to the oxygen content of the water, but in sealed receptacles ZoBell and Anderson[2] found that oxygen was not the controlling factor. They noted a direct relationship between the area of solid surface exposed to the stored water and the bacterial activity in it.

In continuing these studies, freshly collected sea water was filtered by gravity through a Buchner 4G sintered-glass filter into a 50-liter bottle. After shaking the bottle to mix the water and to insure its complete oxygenation, the water was siphoned into glass-stoppered bottles varying in capacity from 10 cc. to 10,000 cc., which were stored in a waterbath at 16°C. The bacterial population

[8] Allen, W. M., and Corner, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 403.

[1] Waksman, S. A., and Carey, C. L., *J. Bact.*, 1935, **29**, 531.

[2] ZoBell, C. E., and Anderson, D. Q., *Biol. Bull.*, 1936, **71**, 324.

[3] Whipple, G. C., *Tech. Quart.*, 1901, **14**, 21.

was determined immediately and at 24-hour intervals thereafter by plating procedures. The oxygen-content was estimated by the Winkler method on duplicate bottles of water. Representative findings are summarized in Table I which gives the maximal bacterial population found in each volume of stored water and the oxygen-content after 20 days' storage.

TABLE I.

The oxygen-content of sea water stored at 16° C. in glass-stoppered bottles of different capacities after 20 days and the maximal bacterial population (3 to 5 days) in similar bottles. The water initially contained 5.46 cc. of oxygen per liter and 231 bacteria per cc.

Volume of sea water	10 cc.	100 cc.	1000 cc.	10,000 cc.
Oxygen per liter	2.59 cc.	2.90 cc.	3.68 cc.	4.17 cc.
Bacteria per cc.	1,475,000	1,080,000	673,000	382,000

The data in Table I show that the greatest consumption of oxygen occurs in the smallest volumes of stored sea water in which are also found the most bacteria. However, the maximal bacterial populations appear within 3 to 5 days, whereas the daily determination of oxygen reveals that the most rapid consumption occurs after the 5th day or, at a time when the bacterial population of the water is rapidly receding. Although the bacterial population of the water may drop to only a few thousand per cc. after 10 days, the rate of oxygen-consumption continues unabated until after the 12th to the 20th day. This indicates that there are many more bacteria respiring than the number which are demonstrated by plating procedures, or else there is something in the water besides the bacteria which causes the consumption of oxygen. Several different kinds of experimentation prove that the consumption in stored sea water after the plate-count decreases is caused by the respiration of periphytic bacteria which have attached themselves so firmly to the walls of the glass receptacle that they are not dislodged by vigorously shaking the bottle prior to sampling.

First, as reported above, the most rapid consumption of oxygen occurs in sea water stored in the smallest receptacles which present the largest solid-surface area per unit volume of water. Further increasing the solid surface by the addition of glass beads, glass rods or silicious sand accelerates the rate of oxygen-consumption although available organic matter limits the total amount of oxygen consumed. The larger surface permits the attachment of more periphytic bacteria and shortens the distance between the solid surface and each unit of water containing the nutrients and oxygen.

Second, when the sea water is carefully siphoned after the 10th

day of storage into another receptacle filled with nitrogen to prevent absorption of oxygen from the air, oxygen-consumption is greatly retarded and becomes proportional to the number of bacteria in the water. However, when the original container is refilled with sterile sea water, oxygen-consumption begins almost immediately and at approximately the same rate as when it was interrupted by the removal of the initial water. This is interpreted as showing that the majority of the respiring bacteria are tenaciously attached to the walls of the container. Fresh water bacteria have a similar tendency to adhere to submerged glass slides.[4] Henrici[5] recommends the term "periphytic bacteria" to describe those which grow attached to submerged surfaces rather than the term "attachment bacteria" which ZoBell and Allen[6, 7] applied to marine bacteria which they found growing on glass slides submerged in the sea.

Third, the direct microscopic observation of slides submerged in different volumes of stored sea water reveals a relationship between the number of periphytic bacteria attached to the glass and the oxygen consumption—assuming the bacteria are evenly distributed over the entire solid surface of the receptacle as on the glass slides. This is illustrated by the data in Table II. While there are more bacteria

TABLE II.

Bacteria per cc. of sea water, oxygen-content per liter and bacteria per sq. cm. of glass slide submerged in different volumes of sea water after 14 days at 16° C. The solid surface offered by each receptacle including the slide and the ratio of the total volume to the solid surface are also given.

Volume of sea water	120 cc.	1225 cc.	13,220 cc.
Area of solid surface	168 sq. cm.	660 sq. cm.	3194 sq. cm.
Ratio of cc. : sq. cm.	1 : 1.14	1 : 0.54	1 : 0.24
Bacteria per cc.	216,000	34,000	62,000
Oxygen per liter	3.53 cc.	4.38 cc.	4.71 cc.
Bacteria per sq. cm.	12,900,000	30,500,000	43,600,000

per unit area of solid surface in the larger receptacles, there are not as many periphytes per unit volume of water as in the smaller re-receptacle. This is attributed to the closer proximity of the water to the solid surface in the small receptacles. More periphytes per unit area are supported by the greater amount of nutrients in the larger volumes of sea water but the distance of these nutrients from the periphytes renders them less available.

The concentration of nutrients in sea water limits the activity of

[4] Henrici, A. T., *J. Bact.*, 1933, **25**, 277.

[5] Henrici, A. T., *J. Bact.*, 1936, **32**, 265.

[6] ZoBell, C. E., and Allen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1409.

[7] ZoBell, C. E., and Allen, E. C., *J. Bact.*, 1935, **29**, 239.

bacteria therein.* When the solid surface in stored sea water is increased tremendously by the addition of silicious sand or inert colloidal substances, the total bacterial population reaches only 10 to 100 million per cc. including estimated periphytes. Upon the addition of a little organic matter it greatly exceeds this number regardless of the volume of the receptacle in which the water is stored or the area of solid surface. However, when more than 10 mg. of nutrient material such as peptone is added per liter of sea water, the proportion of periphytes which develop is decreased. In sea water containing more than 100 mg. of peptone per liter the beneficial effect of solid surfaces is masked by the great abundance of bacteria which appear in the water. Under these conditions bacterial activity in large volumes of water is just as great as in small volumes. ZoBell and Anderson[2] have advanced a theory to account for these phenomena and the studies are being continued.

8936 C

Quantitative Determination of Vibratory Sensibility.

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The determination of the threshold of vibratory acuity is a valuable part of the neurological examination, particularly in the diagnosis of lesions affecting the posterior columns of the spinal cord. There have been numerous studies in which an attempt has been made to obtain a quantitative measure of acuity. The tests have all been made with a tuning fork of some description, usually with some device designed to measure the intensity of the vibration, an end which, in the opinion of the workers themselves, was not satisfactorily attained.

We have, therefore, constructed an instrument which produces a vibration of constant frequency, the intensity of which can be varied over a considerable range, and measured accurately. It consists of an iron pole-piece which constitutes the vibrating member, and to which is attached a round, metal button 12.5 mm. in diameter which is placed over the bony prominence to be tested. This pole-piece

* According to Krogh and Keys (*Biol. Bull.*, 1934, **67**, 132), natural sea water contains less than 10 mg. organic matter per liter, much of which is not utilizable by bacteria.

is actuated by an electromagnet energized by an alternating current of 60 cycles per second. By means of a potentiometer this current may be varied, and its strength measured by means of an alternating current milliameter in series with the magnet winding. Thus the amplitude of vibration, roughly proportional to the current flowing through the electromagnet, is subject to control and measurement. Since the intensity of vibration bears no absolute relation to the amperage, depending on the design of the coil in the particular electromagnet, this intensity is expressed in arbitrary units from 0 to 10. Each individual instrument should, therefore, be calibrated to correspond to the original one used as a standard. An even pressure is maintained by allowing the instrument to rest by its own weight over the bony prominence, the operator merely supporting the end of the handle. Thus the intensity of the stimulus for any given scale reading is constant from time to time or from individual to individual.

Pearson,[1] who reviewed the literature on this subject, noted a considerable variation of vibratory acuity with age, the threshold increasing markedly after the fifth decade. We have, therefore, determined the vibratory acuity of 125 individuals apparently free of central nervous system disease. Figs. 1 and 2 illustrate graphically the decade averages for vibratory sensibility as determined over the patellae and medial malleoli of these patients. Each number is the average of the appearance and disappearance threshold for

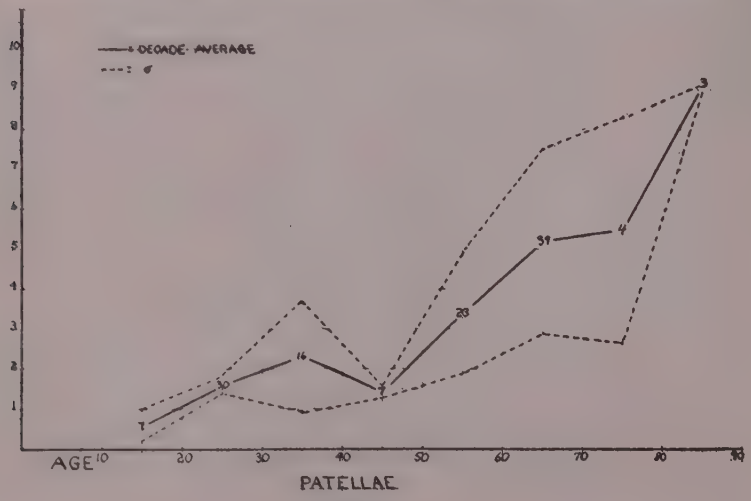

FIG. 1.

[1] Pearson, G. H. J., *Arch. Neurol. and Psychiat.*, 1928, **20**, 482.

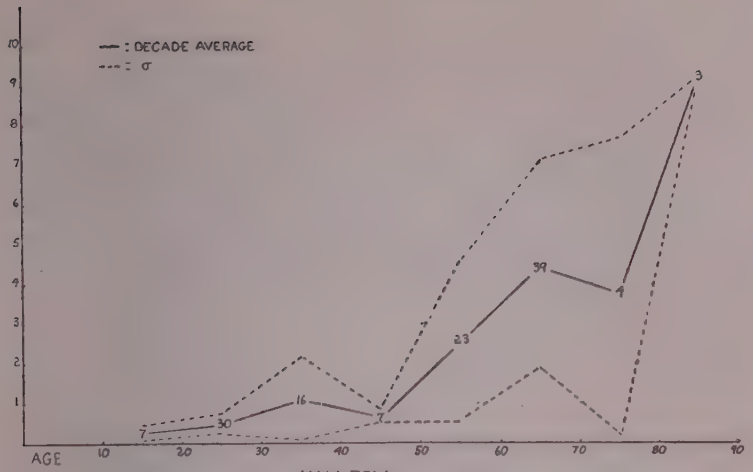

FIG. 2.

both sides. We feel that this average is a more accurate indicator than either the average of the appearance or disappearance threshold taken separately. The field between the broken lines represents the standard deviation computed for each decade average.

A gradual increase in the average threshold with age is apparent. Since the standard deviation also increases with the age of the group, a decrease of vibratory sensibility in the upper decades must be considerable to be of any significance. The 3 individuals over 80 were unable to detect any vibration with our instrument.

We intend to determine vibratory acuity in a much larger number of normal subjects, so that we can, if possible, present more definite limits for the normal range of this sensibility for the different age groups, as a preliminary to the determination of vibratory sensibility in disease. We have already determined the vibratory acuity of 90 patients suffering from arthritis of Ely's type II by this method, but only one age group is large enough to justify a report.

Examination of Figs. 1 and 2 shows the average threshold for normal individuals in the seventh decade to be 4.4 over the medial malleolus and 5.2 over the patella. Similar determinations made on 25 arthritic patients in the same age group showed the value over the medial malleolus to be 6.25 and 6.8 over the patella. The standard error of the difference between normal and arthritic patellae is 0.587, and that between the normal and arthritic malleoli, 0.753. The actual difference between the two means is 1.6 for the patellae, and 1.85 for the malleoli. Since this actual difference is 2.7 times

the standard error of difference for the patellae and 2.4 times that for the malleoli, it probably is significant.

Our finding of increased vibratory acuity threshold with age is in accord with the work of Corbin and Gardner,[2] who found a decrease in the number of myelinated fibers in the spinal roots in man with age and with similar findings of Duncan[3] in the rat. This would seem to imply that arthritic patients have a greater loss of proprioceptive fibers than a similar age group of normals. The possible implication of this finding in the etiology of this type of arthritis must be borne in mind.

8937 P

Effect of Benzedrine Sulfate on Basal Metabolic Rate.*

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The use of benzedrine in narcolepsy has been advocated by several workers.[1, 2, 3] The results have been uniformly excellent, the drug affording complete relief from symptoms; no patients have failed to respond to adequate dosage. In the case-records reported, the basal metabolic rates recorded have averaged well below the normal range. It has been stated that the drug can awaken experimental animals from anesthesia produced by barbital given intraperitoneally.[4, 5] In humans, besides its profound effect in narcolepsy, it has produced also marked cerebral stimulation, insomnia, and rise in blood-pressure; this occurs in normal individuals, likewise. One would expect, therefore, that benzedrine would markedly increase metabolism, most probably indirectly through its stimulative action.

The following study was undertaken to determine the metabolic

2 Corbin, K. B., and Gardner, E., Personal communication.

3 Duncan, D., *J. Comp. Neurol.*, 1934, **59**, 47.

* Assisted by a grant from the Christine Breon Fund.

† Biochemist, University of California Hospital.

1 Prinzmetal, M., and Bloomberg, W., *J. A. M. A.*, 1935, **105**, 2051.

2 Ulrich, H., Trapp, C. E., and Vidgoff, B., *Ann. Int. Med.*, 1936, **9**, 1213.

3 Peoples, S. A., and Guttmann, E., *Lancet*, 1936, **230**, 1107.

4 Alles, G. A., *J. Pharm. and Exp. Therap.*, 1933, **47**, 339.

5 Alles, G. A., and Prinzmetal, M., *J. Pharm. and Exp. Therap.*, 1933, **48**, 161.

activity of benzedrine as shown by the basal metabolic rate. Three subjects were used: T.B.L., a normal white female, aet 28; M.H.S., a normal white male, aet 29; and J.B.L., a normal white male, aet 30. Basal metabolic rates of 2 subjects were determined for 3 successive days, and of the third, for 2 days in order to obtain the true basal levels. Benzedrine sulfate, 20 mg. (2 tablets), was then given for 5 successive days each morning at 9:00 o'clock, following the basal metabolic rate determinations. Therefore, the basal metabolic rate obtained on any given day during the experimental period showed the effect of the benzedrine taken 24 hours previously. The basal rates were not determined on each day of the experimental period, but at the end of the first, second and fifth days of medication. A post-experimental control-rate was determined on the third day (72 hours) after the last dose.

The apparatus used was a Benedict-Roth basal machine (new model). The rates were calculated by the Aub-Dubois standards (body surface). The oral temperature of the subject and the pulse-rate for the second and sixth minutes of the test, were taken—as is the usual routine in the test. Blood-pressures were taken with a mercury manometer by the auscultatory method until 3 comparable systolic pressures were obtained. This was done under the basal conditions mentioned above.

TABLE I.

Date	T.B.L. BMR	Temp.	Pulse	J.B.L. BMR	Temp.	Pulse	M.H.S. BMR	Temp.	Pulse
Sept. 1936	%			%			%		
21	— 8.0	36.8	68-66	—16.0	36.5	70-66	—29.0	36.4	60-62
22	— 7.5	36.7	76-74	—22.0	36.4	58-60	—22.0	36.2	60-60
23*	—	—	—	—24.2	36.4	64-	—35.5	36.2	60-58
24*	—11.0	37.0	68-66	— 6.4	36.4	66-68	+ 0.6	36.5	64-64
25*	—12.6	36.7	70-76	— 7.0	36.4	72-70	— 3.0	—	62-64
26*	—	—	—	—	—	—	—	—	—
27*	—	—	—	—	—	—	—	—	—
28	—13.5	36.7	66-62	—21.0	36.2	68-66	—29.0	36.2	64-64
29	—	—	—	—	—	—	—	—	—
30	—24.0	36.6	88-66	—24.5	36.1	62-66	—44.0	36.1	60-62

*Benzedrine was given on these days after the basal metabolic rate was determined.

Table I gives the basal metabolic rates for the normal and experimental periods, and shows the relationship to the benzedrine dosage. T.B.L. is accustomed to having a basal metabolic rate taken every 5 or 6 months, which had shown a basal level of 13.0% (± 2%) minus. This subject had a slight cold, but without fever, during the test, which may account for the slightly higher level.

It is important that each subject rested from 45 to 60 minutes

before each determination, and that each one felt that no disturbing factors influenced the test, as complete physical and mental repose had been attained.

The results in 2 of the subjects (M.H.S. and J.B.L.) are similar. The first dose produced a definite rise which was maintained after the second dose. Determinations were not made after the third and fourth doses, but the basal rate had dropped to the pre-experimental level after the fifth and last dose. In one (J.B.L.), this basal level was maintained in the post-experimental control determination; in the other (M.H.S.), this determination had dropped sufficiently below the supposed basal level to be significantly beyond the limit of experimental error. The rates of the third subject (T.B.L.) show no significant change except a tendency to drop, though hardly beyond the limit of possible error; in the control determination, however, there was a marked drop, as had occurred in M.H.S. The significance of this drop is not apparent.

There was no significant change in the basal temperature, pulse-rate or blood-pressure of any of the subjects. Although the general effects varied somewhat in the different individuals, they were, on the whole, the same as has previously been reported.[1, 2, 3]

It was not expected that the basal level would be as low as was found. Whether this influenced the rise in rate and whether it accounted for the failure of this rise to be maintained, is not known. It seems reasonable to expect individuals with a normal level (from 10.0% minus to 10.0% plus) to show the same degree of rise; whether the rate would drop to the previous level under continued medication or maintain its level as long as the drug was ingested, is difficult to prophesy. This is the next problem planned for investigation.

8938 P

Clostridium Botulinum Type E.

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Two cultures of *Clostridium botulinum* were sent to this laboratory in March, 1936, by Dr. L. Bier of the Bacteriologic Institute at Dniepropetrowsk, Ukraina, U.S.S.R. Toxin-neutralization tests had suggested a new type. The original cultures were toxic for

mice in doses of 0.001 cc. and antitoxin of types A, B, C and D failed to protect against the the toxin.

The organism is a Gram positive, motile, granular, pleomorphic rod and forms oval, subterminal spores which swell the rods very slightly. In liver-agar shake-cultures small, disc-like colonies with or without small polar fluffs are formed. Colonies on the surface of glucose blood plates are non-hemolytic, greyish, translucent, smooth and flat with a marked tendency toward confluence.

These cultures are not ovolytic and do not liquefy coagulated egg-white even after a month's growth. In beef heart medium there is slight reddening of the meat and a large volume of gas is evolved. Brain is not blackened or digested. In milk, slight acid is produced but the casein is not attacked. Slight liquefaction of gelatin occurred after 23 days. The peptolytic properties are extremely low and the Sörenson figure was 2.00 after 21 days' growth.

Dextrose, levulose, maltose, sucrose, arabinose, xylose and adonite are fermented with little or no gas production in a medium composed of 0.3% Liebig's extract, 0.5% Difco peptone, and 1% carbohydrate. Lactose, rhamnose, galactose, dextrin, raffinose, glycerin, salicin, mannite, inulin and dulcite are not fermented.

The thermal resistance of the spores is extremely low. Spores were destroyed in a suspension in buffer solution (pH 7.4) containing 5 million spores per cc. after heating at 100°C. for 2 minutes or 80°C. for 6 minutes. A suspension containing 50 million spores per cc. failed to show growth after 5 minutes at 100°C. or after 40 minutes at 80°C.

Reciprocal agglutinin-absorption tests showed these 2 cultures to be identical. There was no agglutination with either H or O antisera representative of various groups of *Cl. parabotulinum* and *Cl. botulinum*.

Toxin-production is variable and the cultures tend to become nontoxigenic. Formation of toxin was more constant at room temperature than at 37°C., although growth was equally rapid and luxuriant at either temperature. The most potent toxin obtained was produced in a medium composed of 1% tryptone (Difco) and 2% glucose at pH 7.4.

The MLD of toxin for 350 gm. guinea pigs on subcutaneous injection was 0.02 cc. A 2100 gm. rabbit was killed within 18 hours by subcutaneous injection of 10 guinea pig MLD and 0.025 MLD was fatal to 18 gm. mice within 96 hours. Chickens were not susceptible to injection of 2000 guinea pig MLD. By feeding, approximately 150 times as much toxin was required to kill guinea pigs as by injection and about 200 times as much for mice. Feeding of

1000 guinea pig MLD to 2000 gm. rabbits was without effect. A 3000 gm. monkey showed no symptoms when fed approximately 500 MLD of toxin. Ten days later the same monkey received 2500 MLD and died within 16 hours. A 2500 gm. monkey died within 21 hours when about 2000 MLD were given by mouth. The susceptibility of monkeys to this toxin is somewhat lower than to toxin of *Cl. botulinum* type B which killed in a dose of 100 MLD.

Toxin-antitoxin tests made by injection of mice and guinea pigs showed that antitoxin of types A, B, C and D in doses adequate to protect against 250 to 170,000 MLD of homologous toxin failed to neutralize 2 to 5 MLD of this toxin. Antitoxin for these cultures was produced by injection of rabbits with toxoid and 0.05 cc. protected against at least 10 lethal doses for mice of the homologous toxin. This antitoxin in 0.5 cc. amounts failed to protect against 2 to 3 fatal doses of toxin of types A, B, C and D. Hence, these strains must represent a new type.

The organism closely resembles *Cl. botulinum* types B, C and D in morphology, in failure to attack protein, and in other cultural reactions. The potent neurotoxin acts on small laboratory animals in the same manner as do toxins of other types. It resembles toxins of *Cl. botulinum* types B, C and D in that chickens possess a high degree of immunity to it. Like toxin of *Cl. botulinum* type B it is fatal to monkeys by mouth and thus may play a rôle in botulism in humans. It is not neutralized by antitoxins of types A, B, C or D. The designation *Clostridium botulinum* type E is, therefore, proposed for this organism. Topley and Wilson, Second Edition, 1936, p. 688, have designated the *Cl. parabotulinum equi* of Theiler and Robinson as *Cl. botulinum* type E. Robinson[1] has conclusively shown that the organism of equine botulism belongs to type C and his results were confirmed by Graham and by the authors.

[1] Robinson, E. M., Sixteenth Rep. Director Vet. Serv. and Animal Industry, Union S. Africa, 1930, p. 107.

8939 C

Effect of Adrenocorticotropic Extracts on Accessory Reproductive Organs of Castrate Rats.*

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Clinical[1-7] and some experimental evidence[8-12] indicates a definite but obscure interrelationship between the pituitary, the adrenal cortex and the gonads. It is surprising that a number of experimental workers have been unable to demonstrate a direct interrelationship between the adrenal cortex and the gonads.[13-17] However, Reichstein has recently isolated a male hormone-like substance from the adrenal cortex.[18]

The stimulation of the prostate and seminal vesicles in castrated rats by injections of adrenocorticotropic extracts is herein reported. Adrenocorticotropic extracts were made from whole sheep pituitaries using the HCl-acetone method. The activity of the adrenocorticotropic preparations was tested in hypophysectomized[19] and in

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

1 Broster, L. R., and Hill, H. G., *Brit. J. Surg.*, 1932, **19**, 557.

2 Lisser, H., *Trans. Acad. Am. Phys.*, 1933, **48**, 224.

3 Kepler, Kennedy, Davis, Walters, Wilder, *Proc. Staff Meet. Mayo Clinic*, 1934, **9**, 169.

4 Walters, Waltman, Wilder, Kepler, *Ann. Surg.*, 1934, **100**, 670.

5 Hare, D. C., Ross, J. M., and Crooke, A. C., *Lancet*, 1935, **229**, 118.

6 Cahill, Loeb, Kurzrok, Stout and Smith, *Surg. Gyn. and Obs.*, 1936, **62**, 287.

7 Simpson, E. L., de Fremery, P., and Macbeth, A., *Endocrinol.*, 1936, **20**, 363.

8 Freed, S. C., Brownfield, B., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **29**, 1.

9 Corey, C. L., and Britton, S. W., *Science*, 1931, **74**, 101.

10 Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

11 Corey, C. L., and Britton, S. W., *Am. J. Physiol.*, 1934, **107**, 207.

12 Moskowska, M., *Compte Rend. Soc. de Biol.*, 1935, **118**, 516.

13 Connor, C. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 131.

14 Gaunt, R., and Parkins, W., *Am. J. Physiol.*, 1933, **103**, 511.

15 Simpson, S. L., Kohn-Speyer, A., and Korenchevsky, V., *Lancet*, 1933, **225**, 1194.

16 Howard, E., and Grollman, A., *Am. J. Physiol.*, 1934, **107**, 480.

17 Kutz, R. L., McKeown, T., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 331.

18 Reichstein, T., *Helv. Chem. Acta.*, 1936, **19**, 223.

19 Collip, J. B., Anderson, E. M., and Thomson, D. L., *Lancet*, 1933, **225**, 347.

normal, immature, male rats.[20] Growth, thyrotropic and gonadotropic hormones were absent. When tested in squabs, the extracts were found to contain varying amounts of lactogenic hormone.

Both the testes and epididymes were removed from 21-day-old male rats. Injections were begun on the day following the operation and continued daily over a period of 9 days. The control castrated rats were uninjected. The experimental and control animals were sacrificed on the tenth day after castration. The adrenals and accessory sex glands (prostate, coagulatory gland and seminal vesicles) were removed, weighed, fixed in Susa or Bouin's fixative, and then sectioned and stained for histological examination.

TABLE I.

Extracts			
No.	Total Dose mg.	Adrenal Weights mg.	Weights of Accessory Sex Glands mg.
M141 I	414	59.1	90.4
		79.8	111.5
M152 I	297	66.8	121.0
		55.4	159.8
		42.3	111.3
	603	91.8	120.2
		119.2	106.9
Controls (10 rats) (uninjected)		22.4 (17.4-29.6)	54.8 (39.0-88.0)[21]

The adrenal cortices of the injected rats were greatly stimulated in contrast to the cortices of the controls. The chief changes were the marked hyperemia, increased fat content of the cells throughout the cortex, cellular hypertrophy,[22] and numerous mitoses in the region of the zona glomerulosa.

The prostates of the controls showed a large amount of connective tissue, small tubules, a few acini with cuboidal or low columnar epithelium, and some colloid. The prostates of the treated animals showed evidences of stimulation in that there were larger tubules, more acini, tall columnar epithelial cells with a clear supranuclear zone, numerous mitoses and an increased amount of colloid.[23]

The only evidence of stimulation in the seminal vesicles of treated animals was that the epithelium was slightly higher than that of the controls.[24]

[20] Moon, H. D., (Details of method of extraction and assay to be published).

[21] Korenchevsky, V., and Dennison, M., *Biochem. J.*, 1935, **29**, 1720.

[22] Emery, F. E., and Atwell, W. J., *Anat. Rec.*, 1933, **58**, 17.

[23] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

[24] Moore, C. R., Hughes, W., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 109.

8940 C

Heterogony of the Glutathione Content of New-Born Rabbits.

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Gregory and Goss have presented evidence[1, 2, 3] that the concentration of glutathione in new-born rabbits is correlated with adult body size. The methods of analysis as well as the results are treated exhaustively in the referred publications. However, if positive heterogony (Huxley[4]) exists in the accretion of glutathione, its rate of increase in relation to the rate of increase in body weight may be the same in the several breeds studied and yet give an indication of a correlation such as noted by Gregory and Goss. This point of view may be strengthened by allusion to Needham's theory of a uniform chemical ground plan of growth. Needham[5] presents evidence that the values of the coefficient of heterogonic growth tend to be very similar for the same substances in different species, so that the question arises whether or not the relationships obtained by Gregory and Goss are only a necessary mathematical consequence of positive heterogony of the glutathione. Some doubt is thrown on the question by the fact that the figure for glutathione content of the embryo pig and the rat throughout life as given by Needham indicates a k value of 0.84 and 0.85 respectively, or a condition of negative heterogony.

In order to check this point, the data presented by Gregory and Goss[1] were reanalyzed, so that heterogony constants were made available. Since the range of observations was rather limited, being confined to the normal variation of the breeds around the respective mean birth weights, the values of k obtained are not necessarily absolute values, but should be used only for comparison of the various breeds for the limited range of body weights available. Furthermore, in order to place the several breeds on a strictly comparable basis, only that part of the body weight distribution was used in which all of the breeds were represented, this being the

1 Gregory, P. W., and Goss, Harold, *J. Exp. Zool.*, 1933, **66**, 155.

2 Gregory, P. W., and Goss, Harold, *J. Exp. Zool.*, 1934, **69**, 13.

3 Goss, Harold, and Gregory, P. W., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 681.

4 Huxley, J. S., 1932, Problems of Relative Growth, Methuen & Co., London, 276 pp.

5 Needham, Joseph, *Biol. Revs.*, 1934, **9**, 79.

segment between body weight logarithms of 0.500 and 0.800. The pure breeds available for the calculation were Flemish, Angora, New Zealand Red and Polish.

The crosses reported by Gregory and Goss (Flemish x Polish and New Zealand Red x Angora) were found to be too variable in that segment to yield straight lines when the logarithms of glutathione were plotted against the logarithms of body weight. It should be noted that the glutathione here refers to both glutathione and ascorbic acid contents since the analyses were made before the refinement of the technique permitted the separation of these 2 constituents of the total iodine reducing substance.

The k values were determined by grouping arrays of 0.1 interval in body weight logarithms and the use of the logarithmic regression formula given by Feldstein and Hersh.[6] Table I presents the k values and their standard errors for the 4 breeds with significant differences indicated in bold type.

TABLE I.
k Values of Four Breeds of Rabbits.

Breed	k	Adult Weight	Differences.	
Flemish	1.047±.032	4585-5000	Flem-NZR	—.208±.144
New Zealand Red	1.255±.105	3000-3600	Flem-Ang	.237±.035
Angora	0.810±.014	2480-3060	Flem-Pol	.430±.095
Polish	0.617±.089	1660-1700	NZR-Ang	.445±.106
			NZR-Pol	.638±.137
			Ang-Pol	.193±.090

It may be seen from the table that the k values follow the adult weights with the exception of the Flemish and New Zealand Red reversal. However, this discrepancy is evidently a result of sampling, since the difference in the k values between these 2 breeds is not significant.

Thus an indication that the correlation between glutathione and body weight is a real one is obtained through this method of analysis, confirming the earlier conclusions arrived at by Gregory and Goss.

TABLE II.
Analysis of Covariance of the Logarithms of Body Weight and Glutathione Content.

Source of variance	Sum of squares		Sum of products	Degrees of freedom	Mean square		Correlation coefficient	Regression of GSH on BW
	BW	GSH			BW	GSH		
Total	.954	2.262	1.137	103			.774	1.192
Between means of breeds	.298	1.457	0.545	3	.09933	.48633	.827	1.829
Within breeds	.656	0.805	0.592	100	.00656	.00805	.814	0.902

[6] Feldstein, M. J., and Hersh, A. H., *Am. Nat.*, 1935, **59**, 344.

For a further substantiation of this, an analysis of covariance of the logarithms of body weight and glutathione content was conducted, the results being presented in Table II.

It should be noted that the F values of the logarithms of body weight and of the logarithms of glutathione content are 15.14 and 60.41 respectively, the corresponding 1% point being 3.98, indicating highly significant differences between the means of the breeds with respect to these 2 variables. The difference in glutathione shows a considerably higher probability of significance than does body weight. Furthermore, a high correlation exists between the two, the fact that the correlation for the total is less than for either between or within breeds, being undoubtedly due to the discarding of fourth place decimals.

However, the significant fact apparent from the table is that while the regression of glutathione on body weight is only 0.902 within breeds, it is 1.829 between means of breeds. This indicates that for every unit of change in the logarithms of body weight within breeds, an increase of only 0.902 obtains in the logarithm of glutathione, while for every unit increase of logarithm of body weight between breeds, a change of 1.829 occurs in the logarithm of glutathione content. The inevitable conclusion is that each of the breeds has its characteristic glutathione content as well as a characteristic rate of change of this factor, which is correlated with the definitive adult size of the 4 breeds studied.

8941 C

Streptococcus Anticoagulant.*

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It was shown by Neter and Witebsky[1] that many bacterial species which produce no demonstrable fibrinolysin in veal-infusion broth do produce fibrinolytic factors if grown in the same medium plus 0.4-2% glucose. They found that in this glucose broth many fibrin-

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED.,1936, **34**, 549, 858.

olytic bacteria often produce 2 fibrinolysins; *S. hemolyticus,* for example, producing: (a) the highly specific Tillett-Garner fibrinolytic enzyme and (b) a hitherto undescribed, relatively non-specific lytic factor. The new lytic factor prevents the coagulation of human plasma, a property rarely demonstrable with the Tillett-Garner enzyme.

We have repeated their work, using the isolated-fibrin technic[2] in place of their relatively crude plasma-clot technic. Confirming their results, we have found that many apparently non-fibrinolytic strains of *S. hemolyticus* will produce anticoagulants if grown in veal-infusion broth plus 0.4% glucose. Demonstrably fibrinolytic strains produce this anticoagulant in addition to a normal amount of the routine Tillett-Garner fibrinolysin. Unlike the fibrinolysin, the anticoagulant is not specific for human fibrin; but will also prevent the clotting of isolated fibrinogen-thrombin-complex from rabbit, sheep, cow and domestic swine. The anticoagulant is not neutralized with concentrations of commercial streptococcal antiserum sufficient to neutralize the specific fibrinolysin. The anticoagulant and fibrinolysin are apparently independent variables in different streptococcal strains.

Chemical differences between the anticoagulant and the routine fibrinolysin are demonstrable by the enzyme-concentration technic of Tillett and Garner. From 24-hour glucose-broth cultures of relatively high anticoagulative titers, purified fibrinolysin is obtained by alcohol (75%, ice-cold) precipitation. Even in a 10-fold concentration this purified fibrinolysin is without demonstrable anticoagulating effects. The anticoagulant remains in solution in the supernatant alcohol, from which it can be recovered by evaporation. The anticoagulant thus recovered is thermostable. It resists heating to 100°C. for 30 minutes. The purified fibrinolysin is destroyed quantitatively if heated to 60° C. for 30 minutes.

There is as yet no evidence that the streptococcal anticoagulant produced in glucose broth is of clinical interest. The fibrinolysin of Tillett and Garner, however, has been demonstrated in clinical lesions.[3]

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[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 495.

[3] Neter, E., and Witebsky, E., *J. Bact.*, 1936, **31**, 77.

8942 C

Spectrographic Analyses of Human Spinal Fluid.*

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In connection with other studies on inorganic salt and metal distribution in cells and tissues we have had occasion to examine spectrographically a series of samples of spinal fluids taken from the usual hospital and clinic population. Our attention was directed toward a search for elements that are not usually recorded as being normal constituents of human spinal fluid. These are Pb, Al, Ba, Sr, B, Sn. Samples of spinal fluid were generously provided for us by Drs. A. F. Hartman and W. B. Wendell.

The method used routinely in our examinations was as follows: 2 cc. of spinal fluid were placed on a carefully cleaned glass plate and evaporated to dryness at 100°C. The residue was scraped together, placed on a pure carbon electrode, wetted slightly with a small drop of the original fluid and dried. The loaded carbon was placed in front of the slit of a Bausch and Lomb Medium Quartz Spectrograph and the salt ignited by means of an intermittent arc. About 100 flashes of the arc covering a total time interval of 80 seconds sufficed to produce good pictures of lines throughout the ultra-violet spectrum. The intermittent arc is formed by making and breaking electrical contact between 2 vertical electrodes. The upper one has a motor-driven piston-like motion while the lower one is fixed and capped with the sample.

In our search for Al we used 22 samples of spinal fluid. The line used to detect the presence of aluminum was the 3082.16 Å line and as a reference line we employed the conveniently placed 3096.92 Å line of Mg. Those Al lines of greater intensity than that of the Mg were called "strong" and those of much less intensity were designated as "weak". If the 2 lines, Al and Mg, were of approximately equal intensity the Al was referred to as "medium". It is admitted that this method of estimation is a more or less arbitrary one, but is frequently used where more accurate estimates are not necessary. A rough estimation of the actual amount of Al present in the samples yielding the strong lines is one part to 10^8 parts by weight, of fluid.

*Aided by grants from the American Medical Association, the C. M. Warren Fund of the American Academy of Arts and Sciences and the Rockefeller Foundation.

The result of the 22 samples examined for Al are briefly as follows: 5 spinal fluids showed strong lines; 11 were medium, and 4 designated as weak. Thus 20 of the 22 gave a positive Al test and 2 were negative.

As a check on the method and the materials blank runs were frequently made on the electrodes. It so happens that when samples are arced in the fashion outlined above very little of the electrode is consumed and when blank runs of the electrodes are made a great deal of the carbon is used. In spite of this balance in favor of the blank electrodes, we have consistently failed to find Al in them. While this at first would seem to meet the necessary control requirements further consideration shows that a more rigorous control measure would be one in which a "synthetic" spinal fluid was arced. For this purpose we concocted a chemical mixture which approaches as far as possible the same salt content as spinal fluid. The "synthetic" spinal fluid, although made with reagent quality chemicals, when examined spectrographically reveals some Al. The amount is sufficiently great to be comparable with the spinal fluids showing Al lines designated as faint. But we are confident that the Al is in the chemicals used in the mixture rather than in the electrodes. Dee Tourtellotte and Rask,[1] for example, report that a spectrographic analysis of the C. P. reagents in their laboratory showed that 50% of them contained definite traces of Al. That we have not been able to obtain a carbon bearing the "synthetic" spinal fluid free of Al need not throw doubt upon the existence of this metal in human spinal fluid. Both the strong and medium lines are well outside the possible contamination category. Nor do we feel that the instruments used in obtaining the samples contribute anything to the fluid. This has been checked by a number of means.

A series of 18 specimens of spinal fluid was examined for Pb. seven of these were from the Children's Hospital and plumbism was a possible diagnosis. None of this group was finally diagnosed as a case of Pb poisoning; however, one showed evidence of lead deposition in the bones. Of the remaining 11 samples 3 were diagnosed clinically as plumbism.

As in the case of spinal fluids studied for Al the results were divided into 3 categories, strong, medium and weak. Four of the specimens showed strong Pb lines, 2 of these were from the 3 diagnosed clinically as Pb poisoning and 2 from the group of children which were suspected of having plumbism. In 6 of the samples Pb

[1] Tourtellotte, Dee, and Rask, O. S., *Indust. Eng. Chem.*, 1931, **3**, 97.

was found to be present in "medium" quantities and in 5 "faint." Three of the spinal fluids showed no Pb.

Observations for Pb were made on the 2833.07 Å line since it is one of the most sensitive. The amount of Pb was estimated by comparing the intensity of the 2833.07 Å line in a suspect with that of a synthetic solution to which had been added a known amount of $Pb(No_3)_2$. From this comparison it was estimated that the specimens designated as having a strong line contained about 10^{-8} parts of lead by weight.

An examination was made of 18 samples of spinal fluid for Sn. Of the group 5 showed definite evidence of this metal with one specimen catalogued as very strong, one medium and 3 weak. The remaining members of the series, 13 in number, showed no trace of Sn. The occurrence of the sensitive 2839.99 Å line was used as a criterion in the estimations.

In addition to the elements named above the ubiquitous Na, Ca, Mg, Cu, K, and P were always found in relative abundance. Ba, Sr, and B were in all specimens examined. We were hampered in our studies by the fact that the electrodes used contained Fe, Mo, Rb, and Si. These elements have been reported as being found in tissues and one might reasonably expect them in spinal fluid.

While our series of cases is too small to permit us to indulge in statistical treatment it may be said that, within the limits of the sensitivity of the method used, all samples of spinal fluid can be expected to show evidence of Al, Ba, Sr, B; about half of them Pb and a fourth of them Sn.

8943 C

Action of Immune Serum on Meningeal H. Influenzae in vitro and in Experimental Infections.

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Freshly isolated meningeal strains of *H. influenzae* differ from most respiratory strains in their "smooth" colony forms and their virulence for rabbits and mice, and these closely correlated proper-

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ties may be readily lost on subculture.†[1, 2] In this instance virulence seems to be associated with ability of the organism to survive and multiply within the host's tissues, rather than with an "endotoxic" effect. In this laboratory filtrates and heat-killed cultures were found to be relatively innocuous to the test animals. Hence, there is little reason to look for "anti-endotoxic" properties in immune serums, and if such serums possess any protective or therapeutic value, this will probably be dependent on bactericidal action. There is reason to believe that a so-called "immune serum" may have little activity against a smooth virulent strain if the strain used for immunization is one which has become rough and avirulent.[1, 2, 3] In the course of my attempts to develop a product for the treatment of influenzal meningitis, tests were made of the bactericidal activity of various antibody preparations, and the results will be briefly presented.

Through the cooperation of Dr. L. T. Clarke of Parke, Davis and Company, several types of serum from immunized horses were prepared and used in these studies: (1) Felton antibody from a horse immunized against 2 meningeal strains of *H. influenzae*. One of these strains was "smooth" when first used in injecting the horses, but later became "rough". The other was an old "rough" strain. (2) Serum from a horse immunized with a strain kept "smooth" by weekly intracisternal injections into rabbits. (3) Antibody from this serum concentrated according to the "Felton" method. (4) Antibody from this serum concentrated by a method said to conserve both globulin fractions, called "Eureka" by Parke, Davis and Company.

The results of bactericidal studies with these sera are shown in Table I. Guinea pig complement was added for the purpose of enhancing the bactericidal action. A series of dilutions up to 1:100,000 was made from a 24-hour culture of a smooth virulent strain, adjusted to the turbidity of tube No. 3 of the McFarland Nephelometer. With the Petroff-Heuser technic,[4] such cultures were found to contain 1,850,000 organisms per cc. The various immune

† Although the terms "rough" and "smooth" seem unsuitable as descriptions of these variants, they are retained in deference to prevailing usage. Strains which exhibit a mucoid growth on suitable media are called "smooth," while all other strains are designated as "rough," although in most instances the colonies are quite smooth.

1 Pittmann, M., *J. Exp. Med.*, 1931, **53**, 471.

2 Pittmann, M., *ibid.*, 1933, **58**, 685.

3 Ward, Hugh K., and Wright, Joyce, *J. Exp. Med.*, 1932, **55**, 223.

4 Baldwin, Petroff and Gardner, Tuberculosis; Bacteriology, Pathology and Laboratory Diagnosis, Philadelphia, Lea and Febiger, 1927.

TABLE I.

Comparative Bactericidal Activity of Immune Horse Serums in Vitro.

Dilution of culture	Dilution of Serum								Serum
	Conc.	1/10	1/50	1/100	1/500	1/1000	1/10,000	1/100,000	
Conc.	+	+	+	+	+	+	+	+	A
1:10	+	+	—	—	—	—	+	+	Serum from smooth strain, concentrated by Eureka method.
1:100	+	+	—	—	—	—	—	+	
1:500	+	+	—	—	—	—	—	+	
1:1000	+	+	—	—	—	—	—	+	
1:10,000	+	+	—	—	—	—	—	+	
1:100,000	+	—	—	—	—	—	—	+	
Conc.	+	+	+	+	+	+	+	+	B
1:10	+	+	+	+	—	—	+	+	Serum from smooth strain, concentrated by Felton method.
1:100	+	+	+	+	—	—	—	+	
1:500	+	+	+	—	—	—	—	+	
1:1000	+	+	+	—	—	—	—	+	
1:10,000	+	+	+	—	—	—	—	+	
1:100,000	+	—	—	—	—	—	—	+	
Conc.	+	+	+	+	+	+	+	+	C
1:10	—	+	+	+	+	+	+	+	Serum from smooth strain, unconcentrated.
1:100	—	+	+	+	—	—	+	+	
1:500	—	+	+	+	—	—	—	+	
1:1000	—	+	—	—	—	—	—	+	
1:10,000	—	+	—	—	—	—	—	+	
1:100,000	—	+	—	—	—	—	—	+	
Conc.	+	+	+	+	+	+	+	+	D
1:10	+	+	+	+	+	+	+	+	Serum from rough strain, concentrated by Felton method.
1:100	+	+	+	+	+	+	+	+	
1:500	+	+	+	+	+	+	+	+	
1:1000	+	+	—	—	—	—	—	+	
1:10,000	0	+	+	+	—	—	—	—	
Conc.	+	+	+	+	+	+	+	+	E
1:10	—	+	+	+	+	+	+	+	Normal horse serum.
1:100	—	+	+	+	+	+	+	+	
1:500	—	+	+	+	+	+	+	+	
1:1000	—	—	+	+	+	+	+	+	
1:10,000	—	—	+	+	+	+	+	+	
1:100,000	—	—	+	+	+	+	+	+	

Each tube contains 0.1 cc. of the given dilution of culture, 0.2 cc. 1:2 fresh guinea pig serum and 0.2 cc. of the given dilution of serum.

+ = growth.

— = no growth.

0 = test not done.

serums were likewise diluted with normal salt solution. Fresh guinea pig serum was added in a dilution of 1:2. Into a given sterile test tube the following amounts were pipetted: 0.1 cc. of the specified dilution of culture, 0.2 cc. of complement, and 0.2 cc. of the specified dilution of serum. The tubes were incubated for 3 hours at 37°, being vigorously shaken every 5 to 10 minutes. Three drops from a standard capillary pipette were then streaked over the surface of chocolate agar slants, the slants were incubated at 37°C., and the final readings were made after 48 hours.

The immune serums showed definite bactericidal activity. Those made by means of a "smooth" strain were definitely more bactericidal than that made with a "rough" strain. The Neisser-Wechsberg phenomena was observed in tests with the "smooth" serums, while the slight bactericidal activity of normal horse serum, which was apparently due to the added preservative, occurred only when the serum was undiluted.

An attempt was made to study the efficacy of these serums in experimental infections, but the technical difficulties were enormous, and the results can only be considered suggestive. Cisternal puncture in rabbits proved quite difficult, and it was very hard to give an accurately measured dose of organisms by this route, since the inoculum had to be small and concentrated. Moreover, rabbits seemed to vary greatly in their susceptibility to the test organism. However, it may be noted that of 13 rabbits receiving mixtures of "smooth" serum, culture, and complement, only 3 died, while all of the 6 controls died. The results of administration of serum at varying intervals after the infection are not clear enough to warrant detailed presentation. A few of these animals recovered, and it is perhaps noteworthy that the blood cultures of serum treated animals that died were usually negative, even in those treated with "rough" serum, whereas in the controls the cultures were uniformly positive.

8944 C

Post-mortem Changes in Mineral Salt Distribution in Nerve Cells.*

Louis L. Tureen. (Introduced by Gordon H. Scott.)

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An analysis, by microincineration, of the distribution of inorganic salts in anterior horn cells following temporary vascular occlusion of the spinal cord (Tureen[1] brought forward the necessity of examining the post-mortem changes in these elements in similar tissues. It seemed to be especially advisable also because of recent reports on the ash distribution in human nerve cells in various pathological conditions.

Tissues were removed from etherized and bled cats at intervals ranging from 5 minutes to 27 hours. One series of animals was permitted to remain at room temperature; a second series of animals was kept in the ice box at 60° F. for similar periods. Sectioning and incineration were carried out as suggested by Scott.[2] Alternate sections of the series were stained with hematoxylin and eosin as controls. The incinerated sections were studied by dark field illumination.

The findings will be related briefly in two parts, the first of which is the appearance of incinerated anterior horn cells after immediate fixation. The results are in general in accord with those for similar types of material described by Scott[3] and by Patton.[4, 5] The first consideration is to establish a "normal" picture—a task admittedly difficult since there is considerable variation in the appearance of the anterior horn cells even under optimum conditions. In general the ash residue of the well-fixed anterior horn cell is uniformly distributed throughout the cytoplasm. This mineral is in small deposits approximately 1 to 2 microns in diameter. It is in this cytoplasmic ash that the greatest variation occurs. In some cases, for example, the remains of the Nissl substance are clearly discernible, in others not. It is as yet impossible to assign this variation to a

* Aided by grants from the National Research Council, the C. M. Warren Fund of the American Academy of Arts and Sciences, and the Rockefeller Foundation.

1 Tureen, L. L., *Arch. Neur. and Psych.*, 1936, **35**, 798.

2 Scott, Gordon H., *Protoplasma*, 1933, **20**, 133.

3 Scott, Gordon H., *Am. J. Anat.*, 1933, **53**, 243.

4 Patton, W. E., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 195.

5 Patton, W. E., *Am. J. Path.*, 1934, **10**, 615.

definite physiological state of the cell. Some cells show a reasonably dense mineral deposition in the background of the cytoplasm, others will be revealed as having but little of it. The reason for this condition is likewise obscure. However, it is felt that it is not likely to be associated with the technical procedure particularly with fixation. Portions of spinal cord prepared by the Altmann-Gersh frozen dehydration method show the same general characteristics as those fixed in alcohol-formalin.

The nuclear minerals are, as is the case in most other cells, distributed in the same pattern that the chromatin material assumes in stained sections. The nucleolus is represented by a massive ash deposit of size comparable to that body in the stained section. Frequently there is at the periphery of the cell a definite condensation of minerals thought to be largely due to the shrinkage of the cell during fixation.

In contrast to this picture of mineral distribution in the cell fixed immediately after death, we have a series of stages which indicate that there is a more or less progressive loss of inorganic salts with time. The demineralization is more marked and more rapid in the tissues left *in situ* at room temperature than in those that were kept chilled. The post-mortem loss of minerals starts to be noticeable at about 3 hours after death and reaches an equilibrium at 15 to 20 hours. The first noticeable loss of ash is manifested in the cytoplasm which shows progressively less mineral with time elapsed between death and fixation. This loss of mineral continues until it is difficult to distinguish where anterior horn cell leaves off and interstitial tissue begins. The Nissl substance loses its minerals early in the process, about 3 to 4 hours sufficing to make it impossible to identify with certainty. The nucleus resists post-mortem salt loss longer than other parts of the cell. Some specimens showed clearly recognizable nuclei even after 27 hours at room temperature.

The delay of post-mortem salt loss occasioned by the chilling (10°C.) of the bodies in the ice box was interesting in that the changes in the cells lagged behind the room temperature tissues by 7 to 8 hours consistently. That is to say, one could expect to find the same condition in a 7-hour room temperature specimen as in a 14- to 15-hour ice box specimen. It is of some interest, too, that the interstitial cells acquired considerable mineral from the tissue fluid while the anterior horn cells were progressively losing salts. As the nerve cells lost their organic elements the tissue fluid gained them. This exchange apparently ceased at 15 to 20 hours after death at room temperature.

8945 P

Use of a Photo-electric Cell in Respiration Apparatus.*

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In the course of studies on the use of helium in obstructive dyspnea it became evident that varying the pressure of the inhaled atmosphere modified materially the effort required to ventilate the lungs. Inspiration was especially aided by inhaling a helium oxygen mixture under a positive pressure of one to 7 cm. of water in dyspnea such as that encountered in severe asthma.[1] Although moderate positive pressures appeared to have some usefulness in expiration by preventing marked deflation of the smaller bronchi and bronchioles, more marked pressures were found fatiguing. Various pulmotors were tried in which inspiration was achieved by positive pressure and expiration by negative pressure but they had the disadvantage of requiring excessive pressures before the shift from inspiration to expiration was possible. They were adapated to the unconscious rather than the conscious patient.

The basic mechanism we employed was a rebreathing apparatus with positive pressure blowers which forced air through soda-lime and made contact with the patient either with a mouth-piece or a mask. The blower in the inspiratory arm of the apparatus provided positive pressure, whereas the blower in the expiratory arm created a negative pressure, each capable of control and measurement by pressure control valves and gauges. The photo-electric cell was used to operate the solenoid valve which opened and closed depending upon the interruption of a beam of light passing through a photo-tube which contained a delicate vane that swung forward and backward as air was inhaled or exhaled.

The detail of the mechanism is shown in Fig. 1. The light source (F) throws a beam of light through the air current vane (D). The vane may take any one of three positions (a) vertical, when there is no movement of gas in the tube, (b) horizontal and to the left, when gas is moving from right to left as to inspiration, (c) horizontal and to the right, when gas is moving from left to right as in expiration. The van interrupts the beam of light in the expiratory position. During the inspiratory position the beam of light strikes

* This work was supported by a grant from the National Research Council.

1 Barach, A. L., *Ann. Int. Med.*, 1935, **9**, 739 *J. A. M. A.* To be published.

the photo-tube (E) which generates a current that activates a photo-electric relay which in turn sets up a current that operates the solenoid (H), opening the valve (G). The gas mixture employed then passes from 1 to 2 only. In the unactivated position of the solenoid, valve G permits the gas to pass from 2 to 3 only.

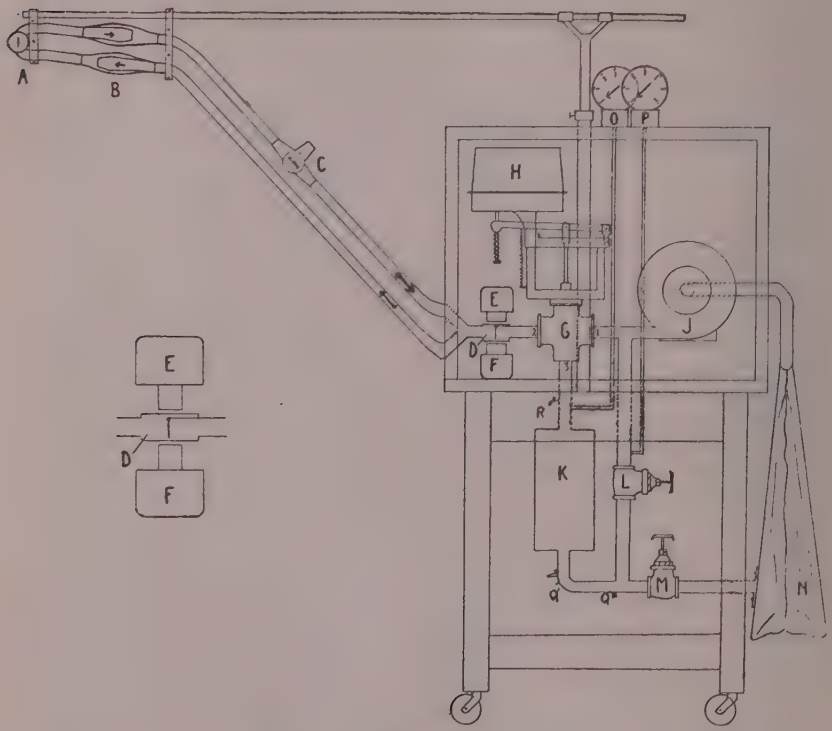

FIG. 1.

A—Mouthpiece holder valve
B—Directional flutter valves
C—3-way by-pass valve
D—Air-current vane
E—Photo tube
F Light source
G—3-way solenoid valve
H—Solenoid
J—Motor blower—high speed 6,000 R.P.M.
K—Soda lime container
L—Inspiratory pressure control valve
M—Expiratory pressure control valve
N—Bag
O—Expiratory pressure gauge
P—Inspiratory pressure gauge

The apparatus may be used for various purposes. To obtain large positive pressures on inspiration and minimal positive pressure on expiration, the patient is attached to the mouthpiece holder A. On inspiration the vane swings in his direction (left horizontal position) and valve G permits gas to pass from motor blower J through the 1-2 path at a positive pressure which can be controlled

by the inspiratory control valve L, and measured by the gauge P. The smaller the opening in the valve the larger will be the positive pressure during inspiration. At the end of inspiration, the vane falls to the vertical position. On expiration the vane swings to the right horizontal position, cutting off the light beam. Valve G is now turned so that the expired air passes through the 2-3 path. In passing through the soda-lime can K, the CO_2 of the expired air is absorbed. The positive pressure on expiration is controlled by valve M. With the valve wide open there is present only minimal pressures incident to the tubing resistance. With the valve partially closed, varying positive pressures may be obtained which can be read on gauge O.

When a positive pressure is desired in inspiration and a negative pressure in expiration both motor blower units must be used (and valve M). The motor blower unit which accomplishes suction is placed in the space Q 1-Q 2. This motor sucks from the soda-lime can K and blows toward the bag. We have been studying this type of mechanism in pulmonary emphysema.

The effects of positive and negative pressures on various types of dyspnea may be studied by this apparatus. The photo-electric cell operates with such speed that there is an exceedingly slight delay in the opening and closing of the solenoid valve, namely, one-tenth of a second. Although the apparatus is a delicate one and may be disturbed by rough handling, it has been used both in the laboratory and on the wards. We have chiefly employed it in severe asthma up to the present but other uses, such as for resuscitation, are being studied.

8946 P

Filtration Studies on Reactive Infusion Fluids.*

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This is a report of attempts to remove by various methods of filtration the reactive agent or agents from infusion fluids known to cause a reaction when injected intravenously. The reaction is characterized in the human being and in the dog by fever, often chills,

* Aided by a grant from the Works Progress Administration.

vomiting and other gastrointestinal disturbances. The fever begins 30 to 45 minutes after injection, reaches its height in 2 to 3 hours, and then begins to recede until in 4 to 6 hours it has fallen to practically normal. In all our experiments in which a leucocyte count was made, there was also found a leucopenia accompanying the reaction reaching its highest intensity 45 minutes after the injection.

Seibert[1] showed that the cause of this febrile reaction in some distilled waters was a gram negative microorganism which has since been called the "pyrogenic bacterium". She further demonstrated that it was not the organism itself, but its products which caused the reaction and that the latter were filtrable through a Berkefeld filter.

In our experiments, 2 lots of reactive water were used, namely, water drawn from the tap and a rather turbid water from a laboratory aquarium. The water was boiled, filtered through fine filter paper and sodium chloride added to make a saline solution of 0.9%. The purpose of adding sodium chloroide was to avoid hemolysis.

The filtration methods used in this investigation were: (1) "dense" Jena filter crucible; (2) Berkefeld filter "W"; (3) Seitz E.K. filter of compressed asbestos; (4) Zsigmondy ultrafilters (gelatinous esters of cellulose) of graded porosity.

Dogs were used as test animals. Richet[2] gives the normal temperature of dogs as 39.25°C. or 102.65°F. In our study of 20 apparently normal dogs, the temperature was found to lie between 100.4°F. and 102.6°F. The temperature of any particular animal was found not to vary over 1.2°F. in the course of 3 hours. Accordingly, a rise of 1.5°F. within 3 hours after injection was taken as a definite fever. The leucopenia mentioned in a foregoing paragraph was also taken as a check on the fever in most of our experiments. In a study of the leucocyte count and its variation in the same 20 dogs, it was found that although the range of the "normal" count is rather wide, the hourly variations are never over 2000. A drop of 5000 or over is therefore considered a leucopenia. In most of the experiments here presented, the drop has been considerably more than this figure.

A typical reaction from tap water and from aquarium water is shown in Experiments 1 and 2 of the table. It will be noted that 45 cc. of the turbid aquarium water in a 16-kg. dog produced more of a reaction than 130 cc. of the clearer tap water.

That the reactive substance is removed neither by a Jena filter

1 Seibert, F. B., *Am. J. Physiol.*, 1923, **67**, 90.

2 Richet, C., *Dictionnarie de Physiologie*, 1885, **8**, 511.

TABLE I.

Each of the experiments in the following table is representative of at least 5 experiments.

Exp. No.	Kind of Water	Type Filter	Wt. of Dog. kg.	Vol. Injected cc.	Temp. Changes °F	Changes wbc. x 1,000	Symptoms
1	Tap		17	130	102.4-104.8		Shivering
2	Aq.		16	45	102.2-105.8	30.65- 6.4	" , diarrhea
3	"	Jena & Berk. W	13.5	150	101.4-105.9		" "
4	Tap	" " " "	14	150	102. -105.2	20.5 - 7.8	" "
5	"	Seitz	15	250	102.2-101.8	17.8 -15.	No symptoms
6	Aq.	"	16.5	250	102.6-101.6	21.2 -20.8	" "
7	"	Zsig. 1 sec.	13	250	102.1-105.3	10.2 - 4.8	Shivering, vomiting, prostration
8	Tap	" 1 "	14	250	102. -104.6	12.8 - 6.4	" , diarrhea
9	"	" 42 "	15	250	101.8-104.2	10.6 - 5.3	"
10	Aq.	" 42 "	16	210	102.4-104.5	40.3 -12.75	" , retching
11	"	" 200 "	16	325	101.8-101.4	17.05-16.8	No symptoms
12	Tap	" 200 "	15.5	350	102.4-102.2	16.4 -14.8	" "

wbc. = leucocyte
Aq. = aquarium
Berk. = Berkefeld "W"
Zsig. = Zsigmondy

crucible nor by the finest Berkefeld (W) is seen in Experiments 3 and 4.

Experiments 5 and 6 show that a Seitz E K. filter removes it, evidence that the reactive agent is adsorbable.

Experiments 7 to 12 indicate that not all membrane filters remove the agent; the coarser filters allow it to pass, while 200 sec. filters retain it. From this it is concluded that retention of the agent by membrane filters is accomplished by sieving rather than by adsorption, and that the agent is of a particulate nature, the size of the particle being larger than the pores of a 200 sec. Zsigmondy membrane filter.

8947 P

Influence of Pathway of Infection on Pathology of Olfactory Bulbs in Experimental Poliomyelitis.

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The purpose of this communication is to describe the lesions produced by the virus of poliomyelitis when it invades the olfactory bulbs from the nose and to indicate their absence when the virus reaches the central nervous system of *Macacus rhesus* monkeys by other pathways. In monkeys succumbing to poliomyelitis after nasal instillation of virus, the olfactory bulbs show changes in the 5 outer layers, *i. e.*, the layer of olfactory nerve fibers, the glomerular, the external granular, the gelatinous, and the mitral cell layers. The lesions in the first 4 layers mentioned appear to be chiefly inflammatory, consisting of perivascular cuffing and diffuse infiltration of polymorphonuclear leucocytes, mononuclears, and lymphocytes. The involved mitral cells undergo necrosis and frequently show neuronophagia by polymorphonuclear and microglial cells.

These changes with some individual variation in extent, were observed in the olfactory bulbs of each of 10 monkeys given the virus by way of the nose, and it should be stressed that although the virus was instilled in both nostrils, the lesions were present, in at least 3 of the 10 animals studied, in only one of the olfactory bulbs. In 12 monkeys which succumbed to poliomyelitis after intracerebral, subcutaneous, or intrasciatic inoculation, examination of both olfactory bulbs revealed no lesions. It is apparent that a study of

the olfactory bulbs may be useful as an indicator of the portal of entry of the virus in experimental poliomyelitis.

Practically no attention has hitherto been paid to the pathology of the olfactory bulbs in human poliomyelitis, and it is believed that their examination in the future should yield data of value to a better understanding of the epidemiology and prophylaxis of this disease.

8948 P

Chemical Studies in Bacterial Agglutination. III. A Quantitative Theory of Bacterial Agglutination.*

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It has recently been shown possible to consider the precipitin reaction as a series of competing bimolecular reactions[1] and so derive from the mass-law an expression

$$\text{mg. antibody N precipitated} = 2RS - \frac{R^2}{A} S^2$$

in which R is the ratio of antibody to hapten or antigen in the precipitate at a reference-point in the equivalence-zone, and A is the amount of antibody-N precipitated at the reference-point. This equation describes closely the behavior of a number of immune precipitating systems.

Since the agglutination reaction may be considered a precipitin reaction at the bacterial surface, it was thought that the above theory might be applied. The test involved the development of an absolute method for the micro-estimation of agglutinin[2] and the use of a single hapten at the bacterial surface and the homologous antihapten. This was realized in a freshly washed, heat-killed pneumococcus I S (Dawson "M") suspension and, for the antibody, Type I anti-pneumococcus horse-serum freed from antibodies other than type-

* The work described in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital, New York City.

1 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **61**, 563; **62**, 467, 697.

2 Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1934, **60**, 643; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 595.

specific anti-carbohydrate by absorption with "C" substance and pneumococcus I R (Dawson "S") suspension.[5] Typical runs are given in Table I and show excellent agreement with the calculated curves and values. Agglutination differs from precipitation in that a maximal N:S ratio is obtained at a relatively small excess of antibody.

TABLE I

Addition of Increasing Amounts of I S Pneumococcus (M) Suspension to 1 ml. of Serum or Antibody Solution.

Bacterial N	Equivalent SI content	Total N pptd.	Antibody N pptd.	Ratio N:S in ppt.	Total N pptd.	Antibody N pptd.	Ratio N:S in ppt.
mg.	mg.	mg.	mg.		mg.	mg.	
		H 701 0.15 N Salt 37°				H 701 0.15 N Salt 0°	
.064	.0166	.254	.18	10.8*	.187	.12	7.2*
.096	.0250	.372	.27	10.8	.290	.19	7.6*
.127	.0330	.476	.34	10.3	.376	.25	7.6*
.191	.0496	.686	.50	10.1	.534	.34	6.9*
.254	.0660	.868	.61	9.2	.722	.47	7.1
.382	.0990	1.060	.67	6.8	1.030	.65	6.5
.517	.132	1.202	.69	5.2	1.246	.74	5.6
.644	.165	1.338	.69	4.2*	1.414	.77	4.7
	Serum Salt	0.008			0.000		
		N = 12.5 S − 34.7 S²			N = 5.8 S − 24.1 S²		
		Maximal S = .114			Maximal S = .182		
		" N = 0.714 calcd.			" N = 0.797 calcd.		
		0.694 found					0.770 found

*Points not considered in calculating equation.
Actual N analyses given to third decimal.
Antibody N values given to nearest second decimal place.

It would appear, therefore, that a quantitative chemical theory has been found capable of accurately describing a typical instance of bacterial agglutination. This theory makes no distinction between the initial chemical combination of multivalent antigen (or hapten) with multivalent antibody and the subsequent flocculation.[6] That this so-called second phase of agglutination is also due to the building up of large aggregates by chemical combination of antibody on the bacterial surface with antigen (or hapten) on the surface of other bacteria is indicated by the following:

Pneumococcus I M suspension is agglutinated (sensitized) with an excess of Type I antiserum, and the organisms are washed free from excess agglutinin with saline. The cells, covered with an excess of multivalent antibody, are easily resuspended in saline. A small quantity of pneumococcus I M suspension, freshly centrifuged and taken up in saline to avoid the presence of dissolved specific

[5] Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1936, **63**, 737.

[6] Cf., for example, Shibley, G. S., *J. Exp. Med.*, 1926, **44**, 667.

polysaccharide, is added to the resuspended agglutinated cells and the mixture agitated for a moment. Reagglutination rapidly takes place and the entire mass of cells falls to the bottom in large clumps. Since this does not occur when pneumococcus II M, III M, or I S (formerly I R) is added, it is difficult to avoid the conclusion that chemical combination of multivalent polysaccharide on the newly added I cells takes place with multivalent antibody on the previously agglutinated and resuspended cells. Thus the entire process of agglutination may be accounted for on a chemical basis, a conclusion already reached by Topley, Wilson and Duncan[5] in a test of Marrack's views.

8949 C

Factors Influencing Nembutal Anaesthesia.

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(Introduced by H. B. Williams.)

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In work recently reported from these laboratories,[1] it was found that the injection of glucose in normal-fed rabbits did not materially shorten the period of depression of nembutal anaesthesia. Fasting for 20 hours increased the duration of the anaesthesia appreciably. There was no correlation between the susceptibility to the drug and the blood sugar levels, either before the administration of the drug or at the time of greatest depression. Although the sugar level was not changed at the time of maximum depression there was a very definite drop in this level at the time of recovery.

Since the drop in the blood sugar level at the time of recovery from nembutal anaesthesia was shown only for normal-fed animals, the question arose as to the reaction of starved animals under the same conditions. It was also necessary to make a more complete study of the changes in the blood sugar level throughout the entire experimental period. The work presented in this paper is an extension of the previous work along these lines.

[5] Topley, W. W. C., Wilson, J., and Duncan, J. T., *Brit. J. Exp. Path.*, 1935, **16**, 116.

[1] Blackberg, S. N., and Hrubetz, M. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 65.

Recovery sugars were done on both normal-fed and starved rabbits. Blood samples were taken at the time of deepest depression and upon complete recovery. The sugar values were determined by the Somogyi Micro Method.[2] Table I again shows the fall in the blood sugar level at the time of recovery for the normal-fed animals. With the starved group the fall obtained was less but since the difference between the initial and final values is more than 3 times the deviation of the difference,

$$\varepsilon_d = \sqrt{\varepsilon^2_{a_M} - \varepsilon^2_{b_M}},$$

its significance is statistically indicated.

TABLE I.
The Blood Sugar Level at the Time of Depression and Recovery from Nembutal Anaesthesia.

	Normal-fed group			Starved group		
	Initial	Anaesthesia	Recovery	Initial	Anaesthesia	Recovery
No. of observ.	136	133	45	44	43	40
Mean	110	110	95	97	101	87
Mean dev.	13	17	10	10	10	16
Mean dev. of mean	1.1	1.4	1.4	1.5	1.5	2.5

In order to ascertain the level of the blood sugar throughout the experimental period, the time curve was made on normal-fed and on starved rabbits. Samples of blood were taken before the injection and at hourly intervals for 6 hours after the injection.

TABLE II.
Time Curve for Blood Sugar Level after Nembutal.
Normal-fed.

		Time after injection in hours					
	Controls	1	2	3	4	5	6
No. of observ.	22	23	23	23	23	23	23
Mean	122	116	104	103	102	105	101
Mean dev.	22	15	11	14	12	11	14
Mean dev. of mean	5.0	3.1	2.3	2.9	2.5	2.3	2.9

TABLE III.
Time Curve for Blood Sugar Level after Nembutal.
Starved 18-24 Hours.

		Time after injection in hours					
	Controls	1	2	3	4	5	6
No. of observ.	22	22	22	22	22	22	22
Mean	105	102	103	96	98	106	107
Mean dev.	6	10	12	12	14	13	13
Mean dev. of mean	1.3	2.1	2.6	2.6	3.0	2.8	2.8

[2] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

From Tables II and III it will be seen again that the normal-fed group showed a significant fall in the sugar level after the second hour. This fall corresponds to the decrease obtained at the time of recovery from the anesthesia, the duration of which averaged 2½ hours after the injection. The starved group again shows a smaller fall, but since the difference between the initial and final values is almost 2 times the deviation of the difference, the change is probably significant. The time at which this fall occurs also corresponds to the time of recovery which, in the starved group, occurred from 3 to 4 hours after the injection.

The drop in the blood sugar of normal-fed rabbits at the time of recovery from nembutal anesthesia brought the blood sugar to the initial level of the animals which were fasted 24 hours. But the normal-fed animals were recovered at the time of the drop in the blood sugar, while the fasted rabbits with the same sugar level remained anesthetized. There is, therefore, no evidence of a correlation between the blood sugar level, *per se*, and the susceptibility to nembutal. It appears that inanition has some effect other than that of lowering the blood sugar. Some of the metabolic processes are altered so as to render the animal susceptible to the drug for a much longer period of time. Since the starved group as well as the normal-fed show a fall in the sugar level at the time of recovery, it appears that the nembutal has some effect upon carbohydrate mobilization. We are at present studying the possible relationship between liver function and this increased susceptibility to nembutal anesthesia.

8950 P

A Contribution to Drug Allergy: Antipyrine.

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Landsteiner and Lampl[1] showed that new protein antigens could be formed through the chemical union between chemically simple drugs, such as anilin, and a protein. This is true for precipitin reactions in rabbits, as well as for shock experiments with guinea pigs (Landsteiner[2]). The sensitized animals do not react to an

[1] Landsteiner and Lampl, *Z. Immun. Forsch.*, 1917, **21**, 193.

[2] Landsteiner, *J. Exp. Med.*, 1924, **39**, 621.

injection of the simple compounds uncombined to protein. On the other hand, they may react to a protein other than the one used for the sensitizing injections, provided this new protein has linked to it the same chemical compound which had been added to the new antigen. It remained to be determined whether the results obtained by precipitin reactions and anaphylactic shock apply to isolated organs as well. For this purpose, experiments were performed with the isolated guinea pig uterus, according to the method of Schultz and Dale.

Antipyrine was diazotized and coupled to protein, after the method of Landsteiner and Lampl. Two antigens were made. In one, the diazo-antipyrine was combined with rabbit serum proteins, and used for injections into rabbits. In another, the diazo-antipyrine was coupled to guinea pig serum proteins, and used for injections into guinea pigs. In this way the interfering presence of antigenic protein was eliminated.

Positive precipitin reactions were obtained against rabbit serum which had been treated with antipyrinediazorabbit protein. Precipitation occurred when the sera were tested to diazoantipyrine-protein antigens of rabbit, guinea pig or egg white proteins. Pure antipyrine added to the precipitin tubes inhibited the precipitin reaction from these antigens.

The uteri of virgin guinea pigs which had been "immunized" to antipyrine-diazo-guinea pig protein were suspended *in vitro,* after the technic of Dale.[3] Wherever it was possible, each uterus was cut into 4 sections, and each segment suspended individually. Each uterus could be subjected to the following procedures:

(a) If no spasm followed the instillation of the antigen, a larger dose was added to the second strip, and more to the third and fourth, until either spasm occurred or the strip designated as insensitive.

(b) If spasm did follow the instillation of the antigen, then the second strip had added to its 100 cc. of bath fluid, 10 mg. of antipyrine, the haptene group.

(c) A third strip of a sensitive uterus was treated with an antigen formed of diazo-antipyrine and a protein other than the one used for the induction of sensitivity.

It was routine to treat a sensitive strip of the uterus, after spasm had been induced, and the Ringer's solution replaced, with a second dose of antigen. The specificity of the first spasm is proven by the lack of effect of the second dose. At the end of each experiment, barium chloride or pilocarpine was instilled into the bath, in order

[3] Dale, *J. Pharm. Exp. Therap.*, 1912, 4, 167.

to test the ability of the uterine strip to respond to an adequate stimulus.

Twenty guinea pigs were injected with antipyrineazoguineapig serum protein antigen. Of these, 15, or 75%, proved sensitive by the uterus method described above. Of the 15 sensitive uteri, 14 were rendered insensitive to the homologous antigen by the previous treatment with 10 mg. of antipyrine added to the 100 cc. of bath fluid. That the antipyrine has a specifically depressant action was shown by the following experiment. Five guinea pigs were injected with eggwhite solution. The uteri were shown to be sensitive in 4 of these. The specific spasm which followed the antigen was uninfluenced by the previous addition of 10 mg. of antipyrine to the bath. *Therefore the previous inhibition of the tissue response by the antipyrine was specific.* This is in conformity with the inhibition of the precipitin reactions by the antipyrine.

None of the strips found sensitive reacted to a second dose of the antigen, proving the specificity of the reaction. The haptene desensitizes *without* previous spasm, while the antigen does so after specific spasm. Desensitization of this tissue cannot therefore be accounted for on a theory of histamine depletion resulting from the spasm.

All of the sensitive guinea-pig uteri responded by spasm also to antipyrine-azo-rabbit serum, or antipyrine-azo-eggwhite protein. In these cases only the azo-antipyrine group had any relationship to the original antigen. These findings are parallel with those obtained with the precipitin reactions in rabbits.

The uteri of 2 of these animals were removed surgically, the animal being allowed to recover. The uteri were sensitive to our antigen. Two days later the surviving pigs were injected intravenously with the antigen. Both animals showed signs of anaphylactic shock. One died within 30 minutes, in typical anaphylactic bronchospasm, as revealed at autopsy. The other survived. Protection experiments in intact pigs was not tried, because the antipyrine proved too toxic to administer in sufficient doses, and for lack of material.

8951 C

Sequence of Medullation of Peripheral Nerves.

Donald H. Barron. (Introduced by J. S. Nicholas.)
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Though the literature contains many accounts of the order in which tracts of the central nervous system acquire their medullary sheaths, Anderson's[1] study of the medullation of the sympathetic system appears to be the only record of observations on the order in which peripheral nerves acquire their sheaths. Stimulated by these observations, a study has been made of the sequence in which systems of fibres in the somatic as well as in the visceral nerves become myelinated in the kitten.

The peripheral nerves of fetal kittens ranging between 8 cm. snout-rump length and full term (c.a. 14 cm.) have been examined either by teasing them out in glycerine after staining for 12 hours in 1% osmic acid, or by sectioning in paraffin, following treatment by the Weigert technic.

The following summary presents the more significant findings in the specimens examined, grouped according to their lengths:

Fetuses 8.0 cm. No medullation was found in any of the peripheral nerves.

Fetuses 9.5 cm. (a) Phrenic nerve contained about 35 faintly medullated fibres. (b) Vagus contained about 12 medullated fibres in the middle of the neck. (c) A few medullated fibres were found in the spinal portion of the eleventh. (d) Medullated fibres were present in all the cervical and dorsal anterior roots. (e) Posterior roots of the cervical segments were medullated only near the ganglion. No medullation was observed on the more caudal dorsal roots.

Fetuses 10.5 cm. (a) The third, fourth, sixth and twelfth nerves contain a few medullated fibres. (b) Medullated fibres were found in both the motor and sensory roots of the trigeminal. (c) None of the fibres of the posterior roots are medullated to their entrance into the cord.

Fetuses 11.5 cm. (a) The phrenic nerve was medullated close to the diaphragm. (b) All of the medullated fibres of the vagus could be traced into the recurrent laryngeal nerve. (c) There was a definite increase in the number of medullated fibres in the nerves which had acquired sheaths earlier.

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[1] Anderson, H. K., Thesis for M.D. degree, Cambridge, 1898.

Fetuses 12.5 cm. (a) Nerves to the sinus hairs were faintly medullated. (b) The fibres in the mesentery were faintly medullated to their entrance into the Pacinian corpuscles. (c) The fibres of the tendon organs were medullated. (d) Medullated fibres were present in the superior laryngeal nerve. (e) The articular nerve of the knee contained 22 medullated fibres.

Fetuses 13.5 cm. (a) There were about 15 faintly medullated fibres in the depressor nerve.

New born. (a) No medullated fibres were found in the papillae of the tongue. (b) Medullated fibres appeared in the pad of the foot but did not reach the epidermis. (c) Medullated fibres reach the necks of the sweat glands but none can be found in the epidermis of the nose.

The phrenic appears to be the first peripheral nerve to acquire extensive medullation; the spinal accessory is next, followed by the recurrent laryngeal branch of the vagus. Of the spinal nerve roots, those of the brachial segments acquire their sheaths first and most rapidly. The sensory nerves to elaborate end-organs all appear to acquire their sheaths at the same time and earlier than the remaining sensory fibres, whose medullation is not complete until after birth.

8952 C

Further Observations on Dorsal Root Components.*

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Additional evidence, both anatomical and physiological, has been introduced by Barron and Matthews[1, 2] to support the presence of efferent components in the dorsal roots. These investigators believe that their histological evidence in the cat, suggests that collaterals of fibers in the posterior funiculi pass to the periphery through the spinal ganglia without cell stations. Such collaterals are said to constitute about 32% of the fibers in the lumbosacral dorsal roots and are of the myelinated variety. The literature bearing upon this

* This investigation was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

1 Barron, D. H., and Matthews, B. H. C., *J. Physiol.*, 1935, **85**, 73.

2 Barron, D. H., and Matthews, B. H. C., *J. Physiol.*, 1935, **85**, 104.

subject was reviewed by Hinsey[3, 4] and, since that time, additional work has been presented by Okelberry,[5] Kahr and Sheehan,[6] and Lugaro.[7]

If approximately one-third of the myelinated fibers in the lumbosacral roots are collaterals of posterior funiculus fibers, it should be possible to demonstrate their degeneration in the distal stumps after section of the dorsal roots, appropriate degeneration times, and staining with the Marchi technic. Hinsey[4] reported that there was no evidence of degeneration of such fibers in serially sectioned Marchi preparations of the distal stumps, ganglia, and peripheral nerves following section of the 6-7 L and 1-2 S dorsal roots in the cat and appropriate degeneration times. He found traumatic degeneration near the point of section but none that passed through the ganglion. This experimental procedure should certainly have shown collaterals of the size which Barron and Matthews illustrate.[2]

We have performed 6 additional experiments bearing on this problem. In young adult cats we sectioned the right 7 L (4 animals), the 6 L and 7 L (1 animal), and the 7 L and 1 S (1 animal) dorsal roots proximal to the ganglia near the spinal cord. Ten days later the animals were killed. The 4-5-6-7 L and 1-2-3 S dorsal root ganglia, with adjoining dorsal and ventral root stumps, were fixed in Müller's solution and stained after the Marchi technic. Each preparation was serially sectioned in the long axis. In addition, various ones of the lumbosacral segments of the spinal cord were fixed, stained, and sectioned.

In the distal segments of the dorsal roots which were sectioned, traumatic degeneration was easily demonstrated in each instance. This degeneration served as an excellent control for the success of our staining procedure. In not one of these preparations was there evidence for any degeneration beyond the traumatic degeneration. In other words, we were unable to demonstrate any degeneration which proceeded through the ganglion. If 32% of the myelinated fibers in these roots were collaterals of posterior funiculus fibers, our section should have severed them from their trophic centers, and typical secondary degeneration should have been present.

Furthermore, when we examined the right dorsal roots of adjacent (4-5-6 L and 1-2-3 S) segments, there was no evidence of the degeneration which might have been expected if the fibers, after

3 Hinsey, J. C., *Quart. Rev. Biol.*, 1933, **8**, 457.
4 Hinsey, J. C., *J. Comp. Neurol.*, 1934, **59**, 117.
5 Okelberry, A. M., *J. Comp. Neurol.*, 1935, **62**, 1.
6 Kahr, S., and Sheehan, D., *Brain*, 1933, **56**, 265.
7 Lugaro, E., *Arch. Suisses de neurol. et de psychiat.*, 1933, **31**, 284.

entering the cord in the 7 L dorsal root, had proceeded up and down the cord in the posterior funiculus with collaterals to adjacent roots. We are forced to conclude that fibers which enter the posterior funiculus of the spinal cord of the cat do not give off myelinated collaterals to the dorsal roots, from either the ascending or descending branches.

Following section of the 7 L dorsal roots degeneration may be seen in March prepartions of the spinal cord as low as the 3 S segment. This indicates that the descending branches may pass as many as 3 segments below their point of entrance. In the upper cervical segments the descending branches may be demonstrated by this method for only one segment below the entrance.[8]

While Okelberry's evidence[5] shows a few myelinated efferent fibers in the dorsal roots of dogs, his own data raise an important question. There is a marked discrepancy in the number of these fibers in Tables I and II (after extradural section), as contrasted with the very few shown in Table III (after intradural section). While Okelberry explains this on the basis of variation in animals, it is very tempting to suggest that, in his extradural sections, he failed to sever scattered neurons of the ganglion from their connections with fibers in the dorsal roots.

In the light of our evidence we are convinced that the antidromic impulses which Barron and Matthews[1] have recorded in the dorsal roots of cats must have some other mediators than myelinated collaterals of posterior funiculus fibers. Lugaro's work[7] substantiates this statement. Furthermore, evidence is available which shows that the number of axons in the dorsal root may equal the number of cells in the ganglion.[9, 10] Such a 1 to 1 ratio would be incompatible with the fibers which Barron and Matthews described.[2]

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8 Corbin, K. B., and Hinsey, J. C., *J. Comp. Neurol.*, 1935, **63**, 119.

9 Ranson, S. W., Droegemueller, W. H., Davenport, H. K., and Fisher, C., "Sensation: Its Mechanisms and Disturbances," *Proc. Assn. Research in Nervous and Mental Disease*, 1935, **15**, 3, Williams & Wilkins, Baltimore.

10 Duncan, D., and Keyser, L. L., *J. Comp. Neurol.*, 1936, **64**, 303.

8953 C

The Serum Protein Complex as a Factor in Regulating Blood Volume.*

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The most important function of serum protein resides in its osmotic attraction for water.[1] This complex is the most effective agent in maintaining the fluid balance between the blood and the intercellular tissue spaces and serous cavities. Thus, Whipple and his associates[2] have reported that intensive and prolonged plasmapheresis results in symptoms resembling those of shock. For the most part, blood volume determinations were not attempted, but the probable explanation of these observed shock reactions is that the reduction of the serum protein concentration prevents the retention of fluid in the vessels in the face of a normal blood pressure. The importance of the colloidal osmotic pressure of the plasma in maintaining a normal blood volume has been emphasized by Stanbury and coworkers.[3] They have demonstrated that the substitution of gum acacia for the serum protein complex results in no obvious disturbances of water balance in the mammalian body. The present communication is intended to throw additional light upon factors regulating blood volume.

In our investigations[4, 5, 6] of the influence of various dietary factors upon the regeneration of serum protein, plasmapheresis was performed quantitatively. Accordingly, the blood volume of the experimental animal was determined periodically as a prerequisite to the calculation of the amount of blood to be withdrawn at each plasmapheresis. The frequency and size of the bleedings were also dependent upon the level of the serum protein concentration, as

* These data form part of a dissertation presented by Daniel Melnick to the Graduate School, Yale University, for the degree of Doctor of Philosophy, June, 1936. The expenses of this investigation were defrayed by a grant from the Research Fund, Yale University School of Medicine.

[1] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, I. Interpretations, Baltimore, Md., Williams and Wilkins Co., 1932.

[2] Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **52**, 54.

[3] Stanbury, J. B., Warweg, E., and Amberson, W. R., *Am. J. Physiol.*, 1936, **117**, 230.

[4] Melnick, D., and Cowgill, G. R., *J. Exp. Med.*, 1936, **64**, 865.

[5] Melnick, D., Cowgill, G. R., and Burack, E., *J. Exp. Med.*, 1936, **64**, 877.

[6] Melnick, D., Cowgill, G. R., and Burack, E., *J. Exp. Med.*, 1936, **64**, 897.

determined daily. In our study, 55 blood volume estimations were carried out on 3 dogs. On these same days the serum protein concentrations, plasma volumes and cell volumes were also recorded. The methods employed have been described elsewhere.[4]

A consideration of some representative values obtained with Dog No. 3 will serve to illustrate the interrelationship observed between serum protein concentration and the blood, cell and plasma volumes.

TABLE I.
Representative Values Obtained with Dog No. 3 During Plasmapheresis Experiments.

Date 1935	Serum Protein Concentration %	Blood Volume cc.	Hematocrit %	Cell Volume cc.	Plasma Volume cc.
3/5	6.82	1335	51.3	684	651
3/21	3.94	1170	44.5	520	650
5/18	3.51	1105	41.3	456	649
5/25	4.47	1242	41.3	513	729
10/18	6.60	1315	44.8	590	725

On 3/5/35 this animal had a normal blood volume of 1335 cc. with a serum protein concentration of 6.82%. A reduction in the serum protein concentration by the technic of plasmapharesis is associated with a drop in the blood volume, as exemplified by the values on 3/21, 5/18 and 5/25/35. In the course of repeated, prolonged plasmapheresis the hematocrit tends to approach anemia levels unless donors' blood cells are injected periodically.[5, 7, 8] It is for this reason that these values show such wide fluctuations which are in no way correlated with the serum protein concentrations. A drop in the cell volume also tends to reduce passively the blood volume. However, when there is a significant reduction in the hematocrit, then the lowered osmotic effect due to a reduced serum protein concentration appears to be negligible in affecting a further reduction in the blood volume, as is evidenced by the constant value for the plasma volumes on 3/5, 3/21 and 5/18/35. Indeed, in some cases where there is a concomitant low serum protein concentration, value of 5/25/35, and a cell volume decidedly less than normal, the plasma volume may actually be greater than the normal. However, with an increase in the serum protein concentration, but with the cell volume constant (determinations of

[7] Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 251.

[8] McNaught, J. B., Scott, V. C., Woods, F. M., and Whipple, G. H., *J. Exp. Med.*, 1936, **63**, 277.

3/21 and 5/25/35), there is a corresponding increase in the plasma volume. Furthermore, with a normal serum protein concentration and a normal blood volume (value of 10/18/35) but with a significant decrease in the cell volume, the plasma volume was found to be somewhat greater than the initial normal value. In other words, *the plasma volume appears to be regulated by the serum protein concentration only insofar as the cell volume is constant. With a significant decrease in the hematocrit resulting in a passive reduction of the blood volume, a lowered serum protein concentration seems to be negligible as a regulatory factor.* This is another example of homeostasis, the ability of the body to resist changes beyond a critical level.

Numerous examples similar to these are recorded elsewhere.[9] These observations should not be confused with those recorded by others[10] with respect to plasma replacement in conditions of primary and chronic secondary anemia. In these pathological conditions, characterized by reductions in both blood and cell volumes, the serum protein concentrations are *normal*.

In searching for some explanation of the maintenance of the plasma volume in the face of a lowered serum protein concentration, the reader is referred to the interesting study conducted by Keys and Adelson.[11] They report that under certain conditions which tend to distort the blood volume, the walls of the capillaries may become impermeable to the so-called "diffusible" calcium ions in the plasma so that an osmotic force is set up in opposition. They suggest that under similar conditions of stress other substances in the plasma may also act as "osmotic buffers."

[9] Molnick, D., "Influence of Diet upon the Regeneration of Serum Protein," Dissertation, Yale University, New Haven, Conn., 1936.

[10] Rowntree, L. G., Brown, G. E., and Roth, G. M., "The Volume of the Blood and Plasma," Philadelphia, Pa., W. B. Saunders Co., 1929.

[11] Keys, A., and Adelson, L., *Am. J. Physiol.*, 1936, **115**, 539.

8954 C

A Stable and Potent Lactic Dehydrogenase Preparation.*

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In investigating some of the biological effects of oxidases, it was found desirable to prepare active enzymes stable enough to keep for some time. Stephenson,[1] in England, prepared a cell-free extract of *B. coli,* containing fairly active dehydrogenases, by lysing a concentrated suspension of the organism in hypertonic buffer solution. Bernheim[2] has also reported the preparation of lactic dehydrogenase from acetone yeast. The product, called "Zymin", gives a fairly active extract and will keep for some time. Gurchot[3] recently described a simple method of extracting lactic dehydrogenase from *Prunus* seeds. This was published without the knowledge that Thunberg[4] had done the same things previously for other seeds.

We have now found a method for making a stable and potent dry preparation of lactic dehydrogenase, 100 mg. of which will decolorize 0.8 mg. of methylene blue per minute in the presence of lactate. This product was obtained by washing ordinary untreated baker's yeast with saline, and then grinding it with phosphate buffer solution saturated with ether, in a ball mill for 8 to 15 hours. This lysate was centrifuged, cooled in the refrigerator, and shaken with one-half its volume of ether. It was then allowed to stand overnight in the refrigerator. A stable ether gel formed, which had no activity, and which could be separated from the remainder of the solution. This served to remove foreign proteins, and cell debris. After 3 such treatments the liquid was filtered and the solution, which was clear and yellow, was saturated with ammonium sulphate. This was allowed to stand in the refrigerator for one hour and then centrifuged. The precipitate so obtained was dried in a vacuum desiccator.

A second extract of enzyme was obtained by grinding the yeast residue with phosphate buffer for 6 hours and allowing it to stand overnight in the refrigerator. This was then centrifuged and treated exactly as the first extract.

The activity of the preparation was estimated by the Thunberg

* Supported in part by the Christine Breon Fund for Medical Research.

1 Stephenson, M., *Biochem. J.,* 1928, **22**, 605.

2 Bernheim, F., *Biochem. J.,* 1929, **22**, 1178.

3 Gurchot, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 285.

4 Thunberg, T., *Skand. Arch. Physiol.,* 1925, **46**, 339.

method, using 1 cc. M/20 sodium lactate, 1 cc. M/2 phosphate buffer at pH 7.6, 2 cc. 1/5000 methylene blue, 100 mg. enzyme powder and 6 cc. of water, making a total volume of 10 cc. The tubes were evacuated for 2 minutes by a Hyvac pump. The reduction time for such tests varied from 30 to 60 seconds, or from 0.8 to 0.4 mg. methylene blue per minute. This dehydrogenase preparation is free from sulphydryl groups as shown by a negative nitroprusside reaction. It has no reducing action on methylene blue when sodium succinate or formate are substituted for sodium lactate. Differences in various other methods of preparation used by other workers result in different final concentrations of enzyme and make a comparison of potency difficult. However, the extract described is probably twice as active as the best previously reported upon by Ogston and Green.[5]

8955 P

Comparative Toxicity of Some Powerful Drugs for the Cat.

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While making a pharmacological and therapeutic study of snake venoms,[1] the writer deemed it desirable to compare their toxicity with that of some other powerful drugs and poisons. To render such a comparative study effective a uniform method of experimentation was required for all the chemicals to be examined. Solutions of the respective substances were accordingly tested for their toxicity by a uniform technique similar to that employed in ouabain and digitalis assay.[2] Healthy cats, weighing from 2 to 3 kg. and kept under light ether anesthesia, were used in these experiments. A cannula was introduced into the femoral vein and a dilute solution of the drug to be tested was injected at the rate of one cc. per 30 seconds until the heart stopped. The drugs examined were atropine sulphate, strychnine sulphate, cocaine hydrochloride, coniine hydrochloride, nicotine alkaloid, aconitine hydrochloride, potassium cyanide, sodium arsenate, cobra venom, rattlesnake venom, ouabain, ricin and abrin. The average lethal dosage of each substance per kg.

[5] Ogston, F. J., and Green, D. E., *Biochem. J.*, 1935, **29**, 1983.

[1] Macht, *Proc. Nat. Acad. Sc.*, 1936, **61**, 22.

[2] Rowntree and Macht, *J. A. M. A.*, 1916, **66**, 870.

weight of cat is shown in Table I. The results obtained with ricin and abrin, however, are not exhibited because the action of these drugs (among the most potent known to man, as little as 0.005 gm. per kg. weight being fatal to a rabbit[3]) is very *slow* and their lethal dosage could not possibly be ascertained by the method described above. The lethal dose of atropine ranged from 30.0 to 42.0 mg.; of strychnine, from 2.2 to 2.8 mg.; of cocaine, from 9.5 to 12.0 mg.; of coniine, from 2.8 to 3.3 mg.; of nicotine, from 1.2 to 1.4 mg.; of aconitine, from 0.25 to 0.31 mg.; of potassium cyanide, from 2.0 to 2.4 mg.; of sodium arsenate, from 180.0 to 195.0 mg.; of cobra venom, from 0.9 to 1.2 mg.; of *Crotalus ruber* venom, from 16.5 to 22.0 mg.; and of ouabain, from 0.09 to 0.12 mg. The figures for ouabain, nicotine, aconitine and cobra venom were carried out to the second decimal place; those for the other drugs tested, to the first place. It will be noted that, with one exception, by far the most potent alkaloid examined was aconitine hydrochloride, 0.28 mg. per kg. weight being the average lethal dosage. Next in potency among the alkaloids was nicotine. When examined by this method, atropine and cocaine salts, on the other hand, gave surprisingly high figures. Snake venoms varied greatly in potency because of their deterioration with age and susceptibility to light, heat, etc. The average lethal dosage of solutions of cobra venom freshly made from a new consignment of the scales was 1.04 mg. per kg. weight while

TABLE I.
Average Lethal Dosage per Kilogram Weight of Cat.

Drug Examined	Concentration	No. of Experiments Performed	Average Lethal Dosage mg.
Atropine sulphate	1: 2,000	5	36.0
Strychnine "	1:10,000	3	2.50
Cocaine hydrochloride	1: 2,500	5	10.0
Coniine "	1:10,000	3	3.0
Nicotine alkaloid	1:10,000	10	1.3
Aconitine hydrochloride	1:10,000	5	0.28
Potassium cyanide	1: 5,000	3	2.2
Sodium arsenate	1: 1,000	2	187.50
Cobra venom	1:10,000	10	1.04
Crotalus ruber venom	1: 5,000	6	20.0
Ouabain	1:10,000	20	0.10

specimens of the same toxin, which had been kept in the laboratory for several years gave a figure as high as 2.6 mg. The oldest specimen of cobra venom, however, was much more potent than even fresh rattlesnake venom. The most interesting finding perhaps was

[3] Sollmann, A Manual of Pharmacology, fifth edition, W. B. Saunders Co., Philadelphia, 1936, p. 224.

that made in connection with a study of ouabain. This glucoside, regarded by physician and pharmacist alike as a medicinal agent and not as a poison, proved to be the most potent substance in a series of compounds studied, 0.1 mg. per kg. weight of cat being its average lethal dose.

It is a truism that every medicinal drug is also a poison; *vice versa*, most poisons under certain conditions exert a remedial action and may be regarded as medicinal agents. The very ambiguous terms by which such poisons are commonly described, however, convey but little scientific information. The word "violent", for instance, may be applied to a very *rapidly* acting poison such as hydrocyanic acid or it may refer to the *minuteness* of the quantity required to produce death (*e. g.*, aconitine, nicotine, ricin). Again, the term "violent" may refer neither to the rapidity of action nor the minute lethal dosage of the drug but may indicate instead the *destructive* local effects or the profound anatomic changes produced thereby. Finally, it is well to note that even the medical man's distinction between "medicine" and poison is often colored by his psychological associations. The words "cobra venom" instantly conjure up the image of a death-dealing serpent while "ouabain" does no more than recall a life-saving heart tonic although it is actually more toxic than the most potent venom listed in the table.

Summary. The comparative toxicity of a series of physiologically potent drugs for cats was determined by repeated injection, under ether anesthesia, of dilute solutions of the respective substances. The results obtained emphasize the ambiguity of the terms commonly used to describe the potency of powerful pharmacological agents. Aconitine, one of the most powerful alkaloidal poisons, is classed as a heart drug; nicotine, another highly poisonous alkaloid, is employed with impunity in the form of tobacco; and ouabain, generally regarded as but a valuable heart tonic, is in point of dosage the most lethal substance the writer has examined and about 10 times as toxic as any specimen of cobra venom tested. In order to convey adequate information with regard to the potencies of pharmacological agents, it is absolutely essential to state accurately the method of assay employed; that is, to specify (1) the species of animal employed, (2) concentration of the drug, and (3) its channel, and (4) speed of administration.

8956 P

Blood Pressure of the Woodchuck and its Response to Injections of Histamine and Epinephrine.

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Opportunity was afforded recently of measuring the blood pressure of the woodchuck. Two fine male specimens weighing 1.7 and 2.5 kg. respectively were obtained for another purpose but before the animals were killed they were etherized. A cannula was inserted into the left femoral artery of each animal and the blood pressure recorded in the usual manner. The initial value for the blood pressure of the larger animal was about 135 mm. of mercury, while that of the smaller animal was about 90 mm. of mercury. As compared with the blood pressure of the rabbit that of the larger woodchuck was considerably higher but the blood pressure of the smaller animal was about equal to that of the rabbit.

Since it is well known that the blood pressure of a rabbit may be elevated or depressed by appropriate doses of histamine given intravenously, I was interested in determining the effect of this amine on the blood pressure of another rodent, such as the woodchuck. Doses of 0.1 mg. of histamine given intravenously to both woodchucks caused a marked fall in blood pressure. After the blood pressure had returned to the control level, another dose of the same size caused a comparable effect.

In response to an injection of 0.1 cc. of 1:1000 solution of epine-

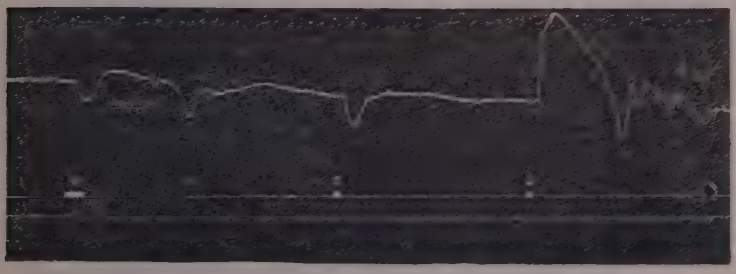

FIG. 1.

From above downward: blood pressure tracing of woodchuck; signal marks and zero line; time in intervals of 5 seconds. At signal mark A, 0.1 mg. of histamine was given intravenously; at B the thorax was compressed; at C, 0.1 mg. of histamine was given intravenously; at D, 0.1 cc. 1:1000 epinephrine was given intravenously.

phrine given intravenously, the value for the blood pressure of each of the woodchucks was raised to about 200 mm. of mercury. There was considerable fluctuation below and above the control level but the blood pressure became stabilized within 4 minutes. At the completion of these observations, the animals were killed (Fig. 1).

8957 C

Carcinoma-Like Proliferations in Vagina, Cervix, and Uterus of Mouse Treated with Estrogenic Hormones.*

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We have referred[1] to the fact that in mice injected with preparations of estrogenic hormones over long periods of time abnormal proliferations of varying degrees of intensity may be induced not only in the mammary gland but also in certain parts of the vagina, cervix, and uterus. In a number of cases conditions were observed which in human beings would be considered precancerous lesions or as changes representing very early stages of cancer. In the monkey, Overholser and Allen[2] noted that in the cervix atypical epithelial proliferations were induced through administration of ovarian hormones. However, these investigators added traumatization of the tissue to the action of the hormones, while in our experiments the tissues were left intact. In two recent publications Lacassagne[3] described adenomatous proliferations of the uterine glands in the rabbit and in the mouse; in some cases the glands penetrated through the muscular layer. Quite recently we have autopsied a mouse in which proliferative changes had progressed further than in any of the others observed. This mouse, of the "Old Buffalo" strain in which spontaneous tumors are relatively rare, had been injected with estrogenic hormones for 24 months, 20 days, beginning at the age of 18 days. During the first 18

* These investigations were carried out with the aid of a grant from the International Cancer Research Foundation.

1 Loeb, Leo, Burns, E. L., Suntzeff, V., and Moskop, M., *Canad. Med. Assn. J.*, 1936, **35**, 117.

2 Overholser, M. D., and Allen, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1322.

3 Lacassagne, A., *C. R. Soc. Biol.*, 1935, **120**, 685, 1156.

months this animal received 10 rat units of theelol in water daily; for the remaining 6 months, 20 days, daily injections of 30 rat units of theelin. At autopsy vagina, cervix, and uterus were very much enlarged. There were adhesions between the vagina and cervix and the surrounding pelvic tissues.

Microscopic examination showed very extensive proliferation in the upper part of the vagina near the cervix, in various parts of the cervix, and in the uterus. The proliferation in the upper part of the vagina consisted largely of squamous epithelium, forming many epithelial pearls containing hyaline material. However, there were also formed irregular ducts lined with columnar epithelium. In the cervix the proliferated tissue consisted largely of ducts lined with cylindrical or cuboidal epithelium, but there were also found some areas consisting of squamous epithelium. In some ducts both kinds of tissues were seen. Higher up in the beginning of the uterus the proliferating tissue consisted almost exclusively of strands or ducts of cuboidal epithelium, and squamous epithelium was rarely noted. These proliferated tissues penetrated deep into the wall of the vagina, cervix, and uterus, and often extended into the subperitoneal connective tissue, and where adhesions existed about the vagina and cervix some of these gland-like strands approached striated muscle tissue and at one point began to enter it. Furthermore some of these abnormally proliferating ducts had pushed their way into certain lymph vessels. Mitoses in this tissue, as a rule, were not numerous except in one area in cylindrical cell strands which took their origin in the uterine mucosa. Here mitoses were frequent and some of them seemed to be hyperchromatic. In various places we found considerable variations in the size of nuclei. In the mammary gland no definite tumor had been produced, but a considerable amount of a type of tissue, the development of which, as a rule, precedes tumor formation, was seen.

We do not believe it is possible at present to designate these abnormal proliferations of the vagina, cervix, and uterus as definitely cancerous, because we cannot be certain that the invasive growth would have continued indefinitely after cessation of injections of estrogenic hormones which served as stimuli in this case. But we may state that if such a condition had been observed in a human being it would have been called malignant.

Of special interest in these observations are the following facts: 1. Long continued application of estrogenic hormones not accompanied by traumatization of the cervix may produce very far going abnormal proliferations not only in the mammary gland, but also

in vagina, cervix, and uterus. 2. Estrogenic hormones may cause proliferations which are not limited to one organ, but affect vagina as well as cervix and uterus. 3. In the mouse observed by us the abnormally proliferating tissue formed under the influence of these hormones was specific in accordance with the actual and potential structures of the various tissues involved.

8958 P

Precipitin Reactions of Helminth Extracts.

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The serological technic of Boyden[1] offers a suitable means of investigating the preciptin reactions of some helminth extracts, and of studying the degree of relationship indicated by the tests. The limited research in this field has been done almost entirely with saline suspensions of powdered worms. The content of these suspensions was unknown, thus prohibiting the use of definite amounts of antigen which in turn prevented comparable interpretation of the results. Schwartz[2] and Hektoen[3] studied the precipitin reactions of a few dried helminths using the best methods available at the time.

In the present work fresh worms were extracted with sterile buffered saline, and the resulting extracts were passed through Seitz filters and bottled under sterile conditions. The antisera were produced in rabbits by injecting intravenously extracts having 0.00384 gm. total nitrogen per kilo body weight; they were divided into 4 doses of increasing amounts on alternate days. None of the rabbits were reinjected. The quantitative precipitin tests, constant in titer within ± one test tube as shown by repeated tests, permit accurate readings of an antiserum with its homologous antigen and with heterologous antigens.

The nitrogen content of the helminth extracts, as determined by the Kjeldahl method, is found to be much less than that of the mammalian blood sera studied by Boyden. He finds, also, that the non-protein nitrogen content of the mammalian sera is negligible,

1 Boyden, Alan, *Am. Nat.*, 1934, **68**, 516.
2 Schwartz, Benjamin, *J. Parasitol.*, 1921, **7**, 144.
3 Hektoen, Ludvig, *J. Infect. Dis.*, 1926, **39**, 342.

TABLE I.

The Titers of Precipitin Tests Expressed as Per Cent Values of the Homologous Titers with their Probable Errors below them. The Values Are Based upon Three or More Tests. Homologous titers in italics.

Antigens / Antisera	Reciprocal							Non-reciprocal	
	Toxocara canis	*Ascaris suum*	*Ascaridia lineata*	*Dirofilaria immitis*	*Macracanthorhynchus hirudinaceus*	*Taenia pisiformis*	*Moniezia expansa*	*Moniezia alba*	*Dipylidium caninum*
Toxocara canis	*100*	12.50 ±0	0.07 ±0.006	0.001 ±0	0	0	0.02 ±0	0	0
Ascaris suum	12.50 ±0	*100*	0.10 ±0	0.05 ±0	0	.03 ±.005	0	0	—
Ascaridia lineata	0	33.33 ±3.75	*100*	0	0	0	0	0	—
Dirofilaria immitis	50.00 ±0	0	0	*100*	0	0	0	0	—
Macracanthorhynchus hirudinaceus	0	0	0	0	*100*	0	0	.31 ±.03	.46 ±.04
Taenia pisiformis	.33 ±.02	.26 ±.02	0	0	0	*100*	0	.15 ±.05	.26 ±.03
Moniezia expansa	0	0	0	0	0	0	*100*	30.03 ±0	—

whereas the opposite is found with the helminths. For example, in extracts of *Dirofilaria immitis,* the heart worm of the dog, and in *Macracanthorhynchus hirudinaceus,* the thorny-headed worm of the hog, the non-protein nitrogen content is apparently as great as the total nitrogen. For these species the total nitrogen is 0.061 and 0.072 gm. per 100 cc. of extract respectively. The biuret and glyoxylic tests for protein are negative to the *D. immitis* extract and only faintly positive to the extract of *M. hirudinaceus.* This would be expected in view of the fact that the latter contains more nitrogen which could involve a larger quantity of protein, provided the extract dilutions were at the limit of sensitivity of the chemical tests. Although the protein content of the worm extracts is low, sufficient is present to stimulate the formation of antibodies having quite high titers.

The data from a series of tests are given in Table I, which shows the degree of relationship of several parasites belonging to the phyla Platyhelminthes and Nemathelminthes. From these results it is noted that in every case the antisera react more strongly with the homologous than with the heterologous antigens. There is a parallelism between the taxonomic relationship of the helminths, as based upon morphological characters, and the precipitin tests which become weaker as the relationship grows less due to the chemical dissimilarity of the extracts. The ascarids react more strongly with each other than with the heart worm or with the cestodes. Interphylar tests occur in the case of 2 tapeworms and 2 roundworms.

There is lack of agreement between the serological and the current morphological relationship of *Macracanthorhynchus hirudinaceus*, the thorny-headed worm of the hog, which commonly is classed with the roundworms. In these tests the antiserum for the thorny-headed worm parasite does not give a single reaction with the nematodes, but gives two reactions with the cestodes. The 2 tapeworm antigens, from *Dipylidium caninum* and *Moniezia alba,* give reactions which indicate a relationship between the Acanthocephala and the Cestoda rather than with the Nematoda.

The principle of reciprocal relationship, which Boyden finds so important when using mammalian blood sera, fails to have significance in the case of the helminths. The complex chemical mixtures present in the whole worm extracts may be the cause for the lack of agreement. Blood sera on the other hand have a simpler structure which would not complicate the reactions as greatly. Agreement is a possibility in the helminths, however, as evidenced in the single case of *Toxocara canis* and *Ascaris suum* which are 12.50% related.

In summary, it is found that both nitrogen and protein concentrations of the helminth extracts are low. Nevertheless when injected into rabbits these dilute antigens are sufficient to produce antisera having moderately high titers. The antisera react more strongly with their homologous antigens than with any heterologous antigens containing an equivalent nitrogen content. The intensity of the precipitin tests parallel in general the systematic position of the species tested, although the principle of reciprocal relationship does not hold. An exception to this parallelism is of interest in that the Acanthocephala reactions indicate a closer affinity to the Platyhelminthes than to the Nemathelminthes.

8959 P

Production of Carcinoma of the Uterus in Mice.

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Spayed and normal young adult female mice were treated twice a week with a .3% solution of 1:2:5:6 dibenzanthracene and a 0.1% solution of estrone* painted on the nape of the neck. Benzene was the solvent for both substances. Treatment with estrone was begun 9 weeks after the treatment with 1:2:5:6 dibenzanthracene because of its more rapid action. Twelve weeks after the treatment with estrone was begun the dose was cut in half because of the development of pyometria. The original dose was estimated to be 125 R.U. Treatment was continued throughout the life of the animals. Six months after the 1:2:5:6 dibenzanthracene treatment was begun there were 27 mice in the group when a mouse died with a large epidermoid carcinoma of the cervix. In the tenth month of the experiment 2 other cases of epidermoid carcinoma of the cervix occurred. These were in the last mouse surviving in the spayed and in the normal group. Both of these mice built nests persistently in the last weeks. All of the animals developed marked hyperplastic, cystic, and metaplastic changes in the breast and uterus. Forty-three percent of the colony developed carcinoma of the breast. Two of the mice developing carcinoma of the cervix had carcinomas of the breast and pyometria. Pyometria and car-

*The estrone was generously supplied by Parke, Davis & Company through the courtesy of Dr. Oliver Kamm.

cinoma of the breast set a pathological limit to the amount of estrone which can be long tolerated. The mouse is not susceptible to spontaneous carcinoma of the uterus. Slye in 39,000 autopsies had only one possible case. No other workers have produced carcinoma of the uterus without direct irritation of the organ, nor have they maintained animals so long on so large a dose of estrone. As we were not able to maintain a group treated with estrone alone, we are limited in analyzing the factors in the production of these carcinomas of the uterus. 1:2:5:6 dibenzanthracene alone painted on the skin does not produce carcinoma of the uterus. Carcinoma of the cervix uteri was produced without direct irritation of the organ by prolonged treatment with massive doses of estrone in conjunction with 1:2:5:6 dibenzanthracene.

8960 P

Observations on the Virus Recovered from 1934-35 Poliomyelitis Epidemic in Los Angeles.

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Attempts were made to recover virus from 19 autopsy cases during the recent epidemic of poliomyelitis in Los Angeles. Seven of these were successful in 1934, four in 1935, as judged by the following results upon inoculation of the emulsified human cord into *Macacus rhesus* monkeys:

(1) Occurrence of pyrexia, roughness of coat, hyperirritability and tremor with subsequent development of paralysis.

(2) Transmission of the virus in successive animal passage of selected strains.

(3) The histopathology was characteristic in that oedema and hemorrhage, perivascular and diffuse infiltration, and necrosis of nerve cells were apparent.

Since the 1934-35 epidemic of poliomyelitis in Southern California has been described as being especially mild in its clinical manifestations with a corresponding low mortality rate and a low residual paralysis rate, it seemed desirable to compare the viruses recov-

* Aided by contributions from President Roosevelt's Birthday Fund, from Manchester Boddy (Publisher), and from Judge Sydney Sanner.

ered from this epidemic with other strains of poliomyelitis virus. To date these Los Angeles strains and the "M.V." strain from the Rockefeller Institute have been compared with reference to symptomatology and pathology produced and comparative immunologic studies have been made in detail with one Californian strain, the "McK" recovered from an autopsy in 1935. Observations include (a) symptomatology as shown by incubation period, temperature changes, type and degree of paralysis, residual paralysis rate and death rate; (b) histopathology produced in the brain and cord, (c) immunity produced and (d) neutralizing substance developed.

Symptomatology. Both pyrexia and paralysis appeared in monkeys on an average of 2 days later with the "McK" strain than with the "M.V." strain. In comparing the paralysis and recovery rate in monkeys infected with these 2 strains, it was found that monkeys inoculated with the "M.V." strain showed a partial paralysis rate of 3.7%, a complete paralysis rate of 96.3%, with 4.7% of the total number recovering, while the monkeys inoculated with the "McK" strain showed a partial paralysis rate of 78%, a complete paralysis rate of 22%, with 84% recovering. The other Los Angeles strains compared favorably in the above characteristics with the "McK" strain.

Histopathology. Although the "M.V." strain and the Los Angeles strains observed in this study exhibit histopathology characteristic of poliomyelitis, monkeys inoculated with the Los Angeles strains of virus show a less degree of cell destruction with comparatively a greater amount of diffuse and perivascular infiltration than was apparent in monkeys inoculated with the "M.V." strain of virus. Similar observations were made by Van Wart, Courville and Hall[1] in their study of the pathology occurring in human autopsy cases during the Los Angeles epidemic.

Immunity. Table I shows the results of cross immunity tests with animals recovered from intracerebral inoculation with Los Angeles strains of virus. It is seen that such recovered animals exhibited an increased immunity upon reinoculation by the intracerebral method with the Los Angeles strain, 100% recovering, while only 84% recovered from first inoculation with the Los Angeles virus. Likewise such animals developed a marked increase of resistance to subsequent intracerebral inoculation with "M.V." virus, 70% recovering while 4.7% recovered from first inoculation with "M.V." virus.

[1] Van Wart, R., Courville, C., and Hall, E. M., *Am. J. Pub. Health*, 1934, **24**, 1207.

TABLE I.
Immunity in Monkeys Recovered from Los Angeles Strains of Virus.

Reinoculation with	No Symptoms %	Slight Symptoms with Recovery %	Total Recovered %
Los Angeles Virus	68	32	100
"M.V." Virus	40	30	70

Neutralizing Antibody. Serum from patients recovered from poliomyelitis acquired during the Los Angeles epidemic neutralized the "McK" strain of virus in 70% of 30 patients tested at a serum dilution of 1 to 20 while at the same dilution it neutralized "M.V." virus in only 30% of the cases tested. It is of further interest to note that serum of monkeys recovered from experimental infection with the Los Angeles strain neutralized "McK" virus in 70% of 30 monkeys tested while it neutralized "M.V." virus in only 40% of the same monkeys.

Variation in the Severity. The foregoing observations on the symptoms and pathology produced by the strains of virus recovered from the recent poliomyelitis epidemic in Los Angeles indicate that they are viruses giving classical symptoms of poliomyelitis in monkeys, differing only in degree from the "M.V." and certain other strains recorded in the literature.

That strains of poliomyelitis virus vary in the symptomatology produced in monkeys is apparent from a review of the results of investigators in this field since some strains are described as "weak" and others as "strong". All workers known by the writers, who have employed the "M.V." strain have recorded severe symptoms and a low recovery rate similar to the findings observed for the "M.V." strain in this study. Jungeblut,[2] using the Aycock strain, finds the severe paralysis rate of inoculated animals high and the recovery rate low, less than 10% (personal communication). Trask and Paul[3] also state that the "Wfd" strain which originated in the Los Angeles epidemic is one of low "virulence—in so far as intracerebral infectiveness is concerned." This is one of the strains used in our current study which we also found to produce mild symptoms in animals. Paul, Trask and Webster[4] further report that the "McC" strain recovered from nasal washings during the Los Angeles epidemic produces mild symptoms in monkeys. All strains from the Los Angeles epidemic, observed by us to date, have been

2 Jungeblut, C. W., *J. Infect. Dis.*, 1936, **58**, 150.

3 Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

4 Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.

found to produce mild symptoms in experimental studies with *Macacus rhesus* monkeys.

Variation in Immunity. Burnet and Macnamara[5] have recorded the existence of immunologic differences between a strain of poliomyelitis virus isolated in Australia and the "M.V." strain.

In the present study it is seen that monkeys recovered from infection with Los Angeles strain of virus demonstrate immunity and neutralizing antibody to both the Los Angeles and the "M.V." virus but that these are higher when tested with Los Angeles virus than when tested with "M.V." virus. Likewise convalescent patients from the Los Angeles epidemic demonstrate a higher neutralizing antibody titre to Los Angeles virus than to "M.V." virus. There is, therefore, demonstrated a definite immunologic relationship between the Los Angeles and the "M.V." strains with, however, minor variations in the degree of immunity produced against local and "M.V." virus.

8961 P

Functional Boundaries in the Sensori-Motor Cortex of the Monkey.*

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The method of local strychninization of the cerebral cortex of the monkey has already established the existence of functional boundaries between the major subdivisions of the sensori-motor cortex, so far as sensation is concerned.

Further study of the problem by the same method, supplemented by recording the action potentials of the cortex, has confirmed the existence of functional boundaries on the "sensory" side and established the existence of such boundaries on the "motor" side.

Strychnine applied locally to any region of the cortex induces in that region typical changes of the "spontaneous" action potentials, notably the appearance of "strychnine-spikes". Though the structural dissimilarity of the various cortical regions precludes the

[5] Burnet, F. M., and Macnamara, J., *Brit. J. Exp. Path.*, 1931, **12**, 57.

* This investigation was aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

attribution of these spikes to any one specific structural element in the cortex, the distribution of the spikes over the cortex differs with difference in architectonic structure of the regions strychninized.

Local strychninization of any one or two square millimeters of area *4* of any subdivision of the sensori-motor cortex "fires" the whole of area *4* of this subdivision and also its postcentral portion. Outside this subdivision no spikes appear.

This is evidenced by the accompanying figure obtained before and after strychninization of one square millimeter of arm area *4*.

Since the action potentials were taken from arm area *4* at a place as far removed as possible (12 mm.) from the locus of strychninization, the figure illustrates the occurrence of spikes throughout this area. Although the electrodes on the face area were only 2 mm.

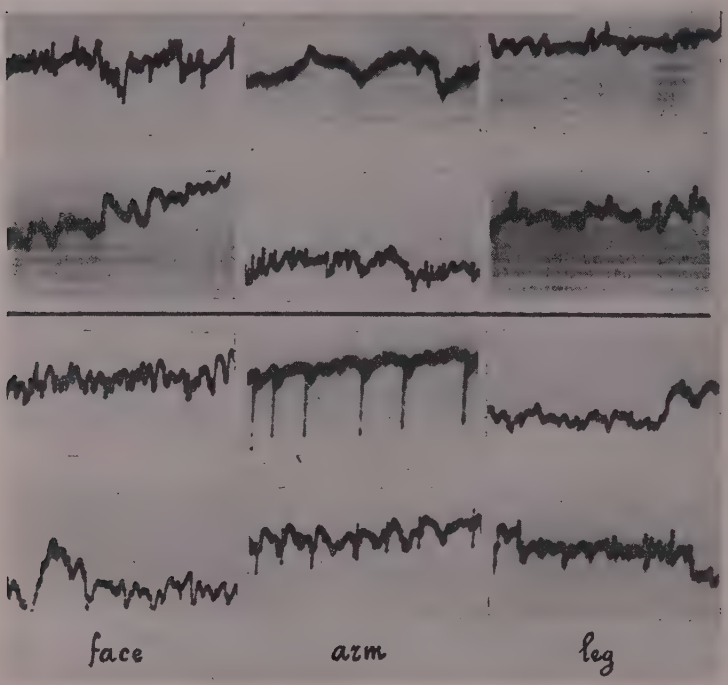

FIG. 1.

Cathode ray oscillograms of action potentials from the precentral and postcentral regions of the face, arm and leg subdivisions of the sensori-motor cortex before (rows 1 and 2 respectively) and after (rows 3 and 4) local strychninization of one square millimeter of the arm area *4*. Note strychnine-spikes in arm area *4* and in postcentral arm area, absence of spikes in face and leg areas.

from those on the arm area, no spikes appear in the record from the face area; and although the electrodes on the leg area were only 2 mm. from the locus of strychninization in the arm area, no spikes appear in the record from the leg area. Distance as such, in area *4*, is insignificant. Functional boundaries determine the findings.

Because strychnine overrides architectonic boundaries while it respects functional boundaries, it has proved a powerful tool in revealing, without disrupting, the functional organization in the intact cortex, by disclosure of differences in functional relations among many of its areas.

This is further instanced by the following observations.

Local strychninization of a small portion of postcentral arm area *1* or *2* "fires" arm areas *1, 2* and *5*, but whereas the strychninization in arm area *2* "fires" arm area *4*, that in arm area *1* decreases the electrical activity of *4*. In neither case is the leg or face subdivision "fired".

A comparable difference exists between the anterior portions of area *6* and its posterior portion, *i. e.*, Hines' "strip" adjacent to area *4*. Local strychninization in the anterior portion of area *6*, whether situated in front of leg area *4* or of arm area *4*, "fires" all the pre- and postcentral portions of the arm and leg subdivisions, whereas strychninization in the "strip" reduces the electrical activity of area *4*.

These findings on area *6* are in harmony with those obtained by extirpation and stimulation of this area (Richter and Hines[1], Bucy and Fulton,[2] Hines[3]).

Studies on action potentials of the cortex and cord and on facilitation of the motor response, both following electrical stimulation of the cortex, have confirmed the existence of the functional boundaries between the major subdivisions of the sensori-motor cortex and given some indication of their significance in the functional organization of the cortex, so far as motion is concerned.

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[1] Richter, C. P., and Hines. M. *Am. J. Physiol.*, 1932, **101**, 467.

[2] Bucy, P. C., and Fulton, J. F., *Brain*, 1933, **56**, 318.

[3] Hines, M., *Am. J. Physiol.*, 1936, **116**, 76.

8962 C

Blood Iron and Copper in Hemochromatosis.

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Hemochromatosis is a rare disease of disturbed iron metabolism usually associated with diabetes and accompanied by cirrhosis of the liver, bronzed pigmentation of the skin, and by the presence of an iron-containing pigment, hemosiderin, and a non iron-reacting pigment, hemofuscin, in the skin, liver, pancreas and various other organs.

Copper has been proposed as an etiological factor in hemochromatosis by Mallory and his coworkers. Mallory, Parker and Nye[1] announced that it was possible to produce pigmentation and cirrhosis of the liver in rabbits and sheep by the administration of copper salts or metallic copper in powdered form. Mallory[2] made a careful study of a series of cases of hemochromatosis among human beings. He pointed out 2 definite factors which he believed had a bearing on the production of the disease, one, the excessive indulgence in alcohol, and the other, occupational contact with copper. In some cases both factors operated. Mallory pointed out that samples of alcohol used in the prohibition era were considerably contaminated by copper. Mallory and Parker[3] repeated their experiments on chronic poisoning with copper and again reached the same conclusions, namely, that as a result of repeated injections of copper over a long period of time a form of pigmentation cirrhosis of the liver was produced.

The results of Mallory and his associates were confirmed by Hall and Butt,[4] but have been denied by Flinn and Von Glahn,[5] by Polson,[6] by Oshima and Siebert,[7] and by Herkel.[8] Mills,[9] after a statistical study of hemochromatosis and diabetes in Koreans who

1 Mallory, F. B., Parker, F., Jr., and Nye, R. N., *J. Med. Res.*, 1921, **42**, 461.
2 Mallory, F. B., *Am. J. Path.*, 1925, **1**, 117.
3 Mallory, F. B., and Parker, F., Jr., *Am. J. Path.*, 1931, **7**, 351.
4 Hall, E. M., and Butt, E. M., *Arch. Path.*, 1928, **6**, 1.
5 Flinn, F. B., and Von Glahn, W. C., *J. Exp. Med.*, 1929, **49**, 5.
6 Polson, C. J., *Brit. J. Exp. Path.*, 1929, **10**, 241.
7 Oshima, F., and Siebert, P., *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **84**, 106.
8 Herkel, W., *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **85**, 513.
9 Mills, R. G., *J. A. M. A.*, 1925, **84**, 1326.

use copper and brass utensils almost exclusively, stated that the rate of incidence of these diseases was less than in the United States. Ramage and Sheldon[10] in a number of chemical and spectrographic analyses of tissues from patients with hemochromatosis have shown the iron content to be tremendously increased over the normal. They have also reported substantial increases in the copper and calcium content of these tissues.

In the light of Mallory's work, we studied the blood copper of 2 male adult patients with hemochromatosis and one male adult patient with a doubtful diagnosis of hemochromatosis. We also determined the blood iron in these 3 patients.

A review of the literature discloses a few scattered figures for the iron content of the blood in cases of hemochromatosis. Garrod and his coworkers[11] reported that in a case of theirs the iron was found to be 48 mg. per 100 cc. of blood. Taking 42 mg. per 100 cc. as the normal blood iron content, they concluded that their figures indicated iron retention in this case of hemochromatosis. Fowell,[12] however, working in the same hospital, had the previous year published figures for the iron content in normal blood ranging from 51 to 55 mg. per 100 cc. with an average of 54.5 mg. His figures for the blood iron content of a case of hemochromatosis were 46 mg. and 52 mg. per 100 cc. of blood. In other reports, Jeanselme[13] recorded a blood iron content of 54.2 mg. per 100 cc. in one case, Howard and Stevens[14] found 45 mg. of iron per 100 cc. of blood in their case, and Cruickshank[15] stated that in a case observed by him there was no increase in the blood iron.

All the investigations cited have been made earlier than 1921. The methods for the determination of iron and the technics employed in these cases may therefore be justifiably questioned. Another factor which makes the data difficult to evaluate is the lack of representative normal blood iron figures as a basis for comparison and the failure to recognize the difference in the blood iron content of adult males and females. With the exception of Fowell, who had a series of 13 determinations on normal individuals, none of these investigators had a normal or control group with which to compare their blood iron findings in hemochromatosis.

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10 Ramage, H., and Sheldon, J. H., *Quart. J. Med.*, 1935, **4**, 121.

11 Garrod, A. E., Gaskell, J. F., Sladden, A. F., Wallis, R. L. M., and Vaile, P. T., *Quart. J. Med.*, 1914, **7**, 129.

12 Fowell, P. H. C., *Quart. J. Med.*, 1913, **6**, 179.

13 Jeanselme, *Bull. et Mem. Soc. med. d. Hop. de Paris*, 1897, **14**, 179.

14 Howard, C. P., and Stevens, F. A., *Arch. Int. Med.*, 1917, **20**, 896.

15 Cruickshank, J., *Brit. Med. J.*, 1921, **2**, 783.

Most of them compared a single determination with a normal reported by another investigator, which may have been estimated by an entirely different quantitative method. On this basis a blood iron determination of 48 mg. per 100 cc. would appear to be high by comparison with the standard of 42 mg. which Garrod adopted, while it would appear low if compared to Fowell's standard of 54.5 mg. For these reasons we feel that our results compared with our normal averages based on determinations in 200 normal adult males are much more significant.

Our findings are presented in Table I.

TABLE I.

Patient	Date Sample Drawn	Time Sample Drawn	Red Cells per Cu. mm.	Hemoglobin,* gm. per 100 cc.	Iron, mg. per 100 cc.	Copper, mg. per 100 cc.
H.	5/26/36	8 AM	4,380,000	11.21	37.56	.148
56 yrs.		4 PM	4,380,000	11.10	37.20	.149
	6/4/36	8 AM	——	12.11	40.56	.145
S.	6/4/36	8 AM	4,100,000	13.26	44.44	.148
48 yrs.		4 PM	4,100,000	12.61	42.24	.130
M.†	5/26/36	8 AM	4,010,000	11.94	40.00	.152
44 yrs.		4 PM	4,010,000	12.44	41.68	.156

*Hemoglobin calculated from blood iron by factor: Mg. of Fe divided by 3.35 = gm. of hemoglobin per 100 cc. Hemoglobin contains 0.335% Fe.[16]

†Doubtful case.

Determinations of copper were made on 5 cc. samples of whole blood by an iron precipitation modification of McFarlane's method[17] using sodium diethyldithiocarbamate. Iron determinations were made by a dry ashing method on 5 cc. samples using potassium thiocyanate reagent. Details of the copper and iron[18, 19] methods have been described previously.

The normal adult male whole blood iron content has been found to average 50 mg. per 100 cc. in a series of 200 determinations previously made in our laboratory.[18, 20] We have found the normal adult whole blood copper to be 0.132 mg. per 100 cc.[18] On the basis of these normal averages, there is no marked increase in blood iron or copper in the cases of hemochromatosis reported here. The blood iron values run from 88.9% to 74.4% of the normal average, and the red cell count from 87.6 to 80.2% of the normal, secondary

16 Butterfield, E., *Z. f. physiol. Chem.*, 1909, **62**, 173.

17 McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

18 Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

19 Fabian, A. A., Sachs, A., and Levine, V. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 662.

20 Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366.

anemia being present in all 3 cases. The low iron values in the blood may be due to the secondary anemia present or may be due to the fact that the deposition of iron in the tissues would tend to reduce the quantity of this element available for the production of hemoglobin. According to Ramage and Sheldon[10] there is also increased copper deposition in certain tissues in hemochromatosis. The absence of a high blood copper content in our cases may be the result of retention in the tissues of this element, but it more likely points to the fact that clinical reports indicate the presence of a mild anemia in hemochromatosis. We have found in general that the more severe the anemia the higher is the blood copper content, especially when there is a marked deficiency both in red cells and in hemoglobin.[18]

The blood copper is slightly higher than the normal average by 9.8 to 18.2%, but yet within the range of normal variation. One determination, however gave a blood copper figure corresponding to the average normal. In the small number of cases reported here the increase in the blood copper above the average figure is not high enough to warrant any relationship in hemochromatosis between the copper in the blood and the abnormal iron metabolism in the tissues.

* We wish to thank Dr. Russell M. Wilder of the Mayo Clinic for permitting us to make copper and iron determinations on blood samples from the three patients mentioned in this article.

8963 P

Enzyme for Decomposition of Creatinine and its Action on the "Apparent Creatinine" of Blood.

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The nature of the substance in filtrates of whole blood and plasma which gives the color with alkaline picrate (Jaffe's reaction) has been for many years a subject of controversy. One group of investigators believe that this material is true creatinine—others deny that creatinine exists in normal blood. Because of the non-specific methods employed for the identification of creatinine, and the very minute quantities of the chromogenic material available in normal blood, it has been difficult for either group to present convincing evidence.

To obtain a definitive answer regarding the nature of the Jaffe-

reactive material in blood, and also to develop a specific method for the analysis of creatinine in biological fluids, an attempt was made to obtain a specific enzyme for creatinine. By means of a technique similar to that described by Dubos and Avery[1] and Dubos[2] it has been possible to isolate 4 different species of soil bacteria with a high degree of adaptability toward a substrate of creatinine. One strain (NC) has been found to grow with unusual ease in a medium of pure creatinine and inorganic salts. When tested under conditions which do not allow cellular multiplication, the bacterial suspension still decomposes creatinine very readily. The enzyme seems to be intimately associated with cellular structure and so far has not been released into solution without destroying its activity. However, when the cells of another bacterial species (HR) are disrupted, the creatinine decomposing enzyme is readily obtained in aqueous solution. At present, the potency of this soluble preparation is low compared with a cell suspension of the NC organisms.

The present crude enzyme preparation (NC) will decompose its own weight of creatinine in 15 minutes at 37° C. and pH 7.0. Its specificity has been tested with certain creatinine derivatives which give the Jaffe reaction.* 5-Methylcreatinine, 4- (or 5-) benzoylcreatinine, 5-benzylcreatinine and 2-benzylcreatinine are not attacked by the enzyme. Acetyl creatinine undergoes a very slight decomposition which does not progress on further incubation with the enzyme preparation. The enzyme preparation also discriminates creatinine from that fraction of the Jaffe-reactive material in human erythrocytes which has been shown by Hunter and Campbell[3] to be different from true creatinine. The power of this material to form a red product with alkaline picrate is not destroyed by the enzyme.

The "NC" enzyme preparation is active in urine and tungstic acid filtrates of blood. We find that it decomposes approximately 50% of the Jaffe- reactive material in human erythrocytes and a much larger fraction in the plasma. This would appear to offer almost conclusive proof of the existence of true creatinine in human plasma and erythrocytes. Data concerning the ratio of creatinine to other substances in blood capable of giving Jaffe's reaction will be given later, together with details concerning the preparation and mode of action of the enzyme.

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[1] Dubos, R., and Avery, O. T., *J. Exp. Med.*, 1931, **54**, 51.

[2] Dubos, R., *J. Exp. Med.*, 1932, **55**, 377; *Ibid.*, 1935, **62**, 259.

* We are indebted to Dr. Isidor Greenwald for the derivatives of creatinine. The nomenclature used is that given by Greenwald, I., *J. Am. Chem. Soc.*, 1925, **47**, 1443.

[3] Hunter, A., and Campbell, W. R., *J. Biol. Chem.*, 1917, **32**, 195.

8964 P

Supervitaminosis C in Tuberculosis.

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Although the effect of subvitaminosis C on the course of tuberculous infection in the guinea pig has been adequately studied, no extensive investigations deal with the possible influence of supervitaminosis. Mouriquand and co-workers,[1] Bieling,[2] Heymann,[3] and Basu[4] showed that the combination of a partial depletion of vitamin C and progressive tuberculous infection apparently shortened the life of guinea pigs. Greene and co-workers[5] demonstrated clearly the effect of vitamin C deficiency on tuberculosis in guinea pigs. On the other hand Leichtentritt[6] gave large amounts of orange juice to tuberculous guinea pigs on normal diets and found the survival period to be twice as long as that of tuberculous animals on a normal diet. Grant[7] reported that increasing the amount of vitamin C seemed to decrease the severity and extent of the tuberculous lesions in the lungs of guinea pigs. Bella[8] noted no increased resistance in tuberculous animals whose diet was supplemented by orange juice or cevitamic acid. Thus, although results reported to date show conclusively that a deficiency in vitamin C is an important factor in the progress of tuberculosis in the experimental animal, no agreement exists among the three authors reporting on the effects of increased amounts of the vitamin above a dosage ample for protection against scorbutic changes.

As none of them produced an extreme degree of supervitaminosis it seemed desirable to test the effect of massive doses of crystalline vitamin C.

Fifteen guinea pigs were maintained on a stock ration consisting of carrots, hay, lettuce and oats. Five of these animals were used

1 Mouriquand, G., Dochaix, A., and Dosdat, L., *Compt. Rend. Soc. Biol.*, 1925, **93**, 901.

2 Bieling, R., *Z. Hyg.*, 1923-24, **101**, 442.

3 Heymann, B., *Klin. Wchnschr.*, 1926, **5**, 59.

4 Basu, N., *Z. Vitaminforsch.*, 1934, **3**, 91.

5 Greene, M. R., Steiner, M., and Kramer, B., *Am. Rev. Tub.*, 1936, **33**, 585.

6 Leichtentritt, B., *Deutsche. Med. Wchnschr.*, 1924, **40**, 672.

7 Grant, A. H., *Am. Rev. Tub.*, 1930, **21**, 115.

8 Bella, G. B., *Bull. Soc. Ital. Biol. Sperm.*, 1935, **9**, 141.

as controls, and the others were given daily intraäbdominal injections of 20 mg. of crystalline cevitamic acid. This amount is considered protective for man against the slightest prescorbutic alterations; it is a massive dose for the guinea pig. The preparation used (Cebione)* was a neutral crystalline product for intravenous administration. The use of more animals was impracticable, because an enormous quantity of Cebione was necessary to maintain even 10 animals over a period of 6 months, which is the length of time necessary to permit the disease to advance to a point at which a comparison can be made. After a 7-day period of treatment with cevitamic acid, both the control and experimental animals were infected by injecting 300,000 H_{37} tubercle bacilli into the groin. All animals showed positive cutaneous reactions to tuberculin 10 days after infection. Daily injection of Cebione was continued for 5 months from the date of infection, at the end of which time each animal had received 30 gm. of cevitamic acid. No detrimental effects of the daily injections were observed; the animals grew at a normal rate and behaved in every way just as the controls. When one of the controls died, all of the remaining animals were killed and necropsied.

Scoring the amount of tuberculosis on a basis of a possible 4 plus for each of 4 organs, lungs, spleen, liver and lymph nodes, averaging these values for each animal and subsequently averaging the values in each group, controls and experimentals, gave a value for controls of 3 plus, while for the experimental supervitaminosis C animals the value was 2.2 plus. No significant differences were observed. Both showed enlargement of inguinal glands, with marked caseation. The spleen, which appears to be the most vulnerable organ, was generally tremendously enlarged and thickly studded with tubercles. The liver was likewise severely affected, and peppered with yellowish necrotic tubercles. The lungs showed less involvement than the other organs.

Two criticisms of this work might be offered. The dose of tubercle bacilli may have been so large that slight influences of the vitamin were obscured. It is also possible that survival periods might have been a more suitable criterion but the limited supply of vitamin C did not permit the injection of a sufficient number of animals to use this basis for a protracted period.

Summary. Supervitaminosis C maintained for a period of 5 months does not protect guinea pigs against subcutaneous injection of 300,000 virulent human (H_{37}) tubercle bacilli.

* Merck & Co., Rahway, N. J., kindly supplied the Cebione used in this experiment.

8965 C

Dynamics of Dissociated Bacterial Cultures.

MICHAEL DOUDOROFF. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, Calif.

In order to obtain material for a study of the alleged specific racial stabilizing or antimutation hormones[1] in bacterial cultures, a 7-day broth culture of *Staphylococcus aureus*, grown from a single agar-plate colony, was plated out on Martin's agar. Plates thus obtained showed from 97 to 99.7% *aureus* colonies with a 0.3 to 3% *albus* dissociation (average dissociation about 1.5%). Pure-line *albus* strains grown from these white dissociates showed no demonstrable tendency to revert to the ancestral orange type. Highly pigmented orange strains similarly selected usually underwent approximately 1.5% *albus* dissociation by the end of 7 days' growth in peptone-broth.

Flasks containing 50 cc. peptone-broth were inoculated with 0.5 cc. of 24-hour broth cultures of pure-line white and orange dissociates thus obtained. The resulting growth-curves of 2 typical contrasting strains are recorded in Fig. 1.

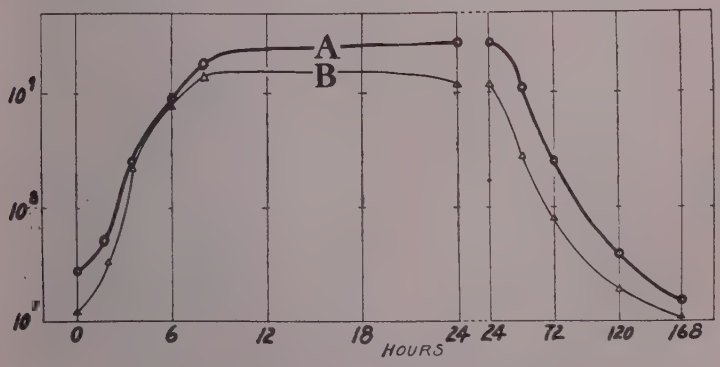

FIG. 1.

Growth of Pure-line Staphylococcal Dissociates.

A, 50 cc. peptone broth inoculated with 0.5 cc. of a 24-hour broth culture of a pure-line orange dissociate. The curve records change in viable count per cc. of broth culture on incubation at 37° C. for 168 hours. The flasks were constantly stirred during the incubation period to prevent sedimentation and the samples thoroughly shaken with glass beads before plating. B, control data in flask inoculated with a pure-line white dissociate.

1 Etinger-Tulezynska, R., *Z. f. Hyg.*, 1932, **113**, 762; Neufeld, F., and Kuhn, H., *Ibid.*, 1935, **116**, 95; Mohr, Werner, *Ibid.*, 1935, **116**, 288.

From this figure it is seen that the *albus* growth-curve is characterized by a shorter lag-phase than the *aureus* control. The *albus* dissociate also showed more rapid proliferation during the logarithmic phase of population increase. Moreover, there was a stabilization of the *albus* viable population at a lower level than in the *aureus* control, and an earlier and more rapid development of the senescent phase, or terminal fall in viable count.

Identically the same relative differences in "growth-vigor" and "longevity" were noted when the same white and orange dissociates were reinoculated into filtrates or centrifugates from 24-hour to 5-day *albus* or *aureus* broth cultures. Varying the volume or the age of the inoculum caused no demonstrable changes in the relative growth rate or longevity.

From the observed differences in inherent "growth-vigor" and longevity one would predict marked fluctuations in the relative percentage of *albus* and *aureus* individuals in a mixed (or dissociated) staphylococcal culture as the culture increased in age. Two graphs confirming the predicted fluctuations are recorded in Fig. 2.

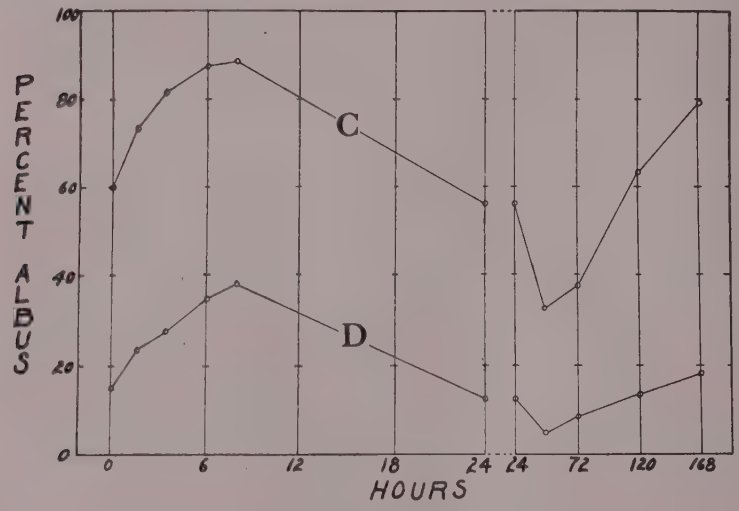

FIG. 2.
Fluctuations in Albus Percentage in Mixed Cultures.

D, 50 cc. peptone-broth inoculated with 15% pure-line *albus* and 85% pure-line *aureus* dissociates. The curve records changes in *albus* percentage during the first 168 hours incubation. C, control flask inoculated with 60% pure-line *albus* and 40% pure-line *aureus* dissociates.

Curve D in this figure records the percentile fluctuations in a mixed *albus-aureus* broth culture, whose initial viable count showed 15% *albus* individuals. The *albus* percentage increased to nearly

40% by the end of 8 hours' incubation (37° C.), then gradually fell to approximately 5% by the end of 36 hours. After the 36th hour the *albus* percentage again rose, reaching about 18% by the 168th hour. Curve C in the same figure records data from a mixed broth culture originally containing 60% *albus* individuals. This curve shows the same type of fluctuations in *albus*-percentage. Identically the same fluctuations were noted when mixed cultures were grown in filtrates or centrifugates from 24-hour to 5-day *albus* or *aureus* broth cultures.

Since all of these fluctuations are predictable from the observed differences in "growth-vigor" and longevity in pure-line *albus* and *aureus* dissociates, such percentile fluctuations furnish no evidence of existence of a specific hormonal antagonism between white and orange dissociates of *S. aureus*.

8966 P

Action of Compounds Related to Cysteine on the Regression of Jensen's Rat Sarcoma.

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From the Division of Pathology, University of California Medical School, San Francisco.

Since the report[1] of the effect of cysteine hydrochloride on Jensen's sarcoma in rats, attempts have been made to determine the active portion of the cysteine structure responsible for the tumor regressing action. This has been studied by injecting into the tissue of the Jensen rat sarcoma several acids with the same pH as the cysteine hydrochloride solutions, fractions of the cysteine molecule, and compounds built up from the cysteine molecule.

In the first group, hydrochloric acid, sulphuric acid, and acetic acid were tried, and also because of its sulphur content rather than acid properties, sodium thiosulphate. The acids were adjusted to the same pH as the solutions of cysteine hydrochloride and were injected in equivalent volumes. All the acids were rapidly absorbed from the tumor tissue. Except for a little central hyperemia in the tumor and occasionally a small focus of necrosis in the center of the tumor mass, no cytological changes were noted. There was no influence

1 Connor, C. L., Carr, J. L., and Ginzton, L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 374.

apparent upon the tumor growth. In the case of sodium thiosulphate no effect whatever was noted.

Because of the similarity between cysteine and alanine, the latter was injected directly into tumor tissue in dosages equivalent to the effective cysteine dose. Since the alanine formula is identical with cysteine, excepting for the removal of the S-H radical, it was felt that any differences in effect might be ascribed to the sulfhydryl group. Whereas 50 mg. of cysteine hydrochloride in 1 cc. of water causes early necrosis and often complete regression of a sarcoma nodule 2.0 cm. in diameter, a similar dose or double the dose of alanine has no effect. In fact, 200 mg. of alanine were tried daily for 5 days in the same tumor and no appreciable effect was noted either upon the tumor or upon the host.

The mercaptan radical was then injected in the form of ethyl mercaptan. Because of its low boiling point the drug was injected quickly, being withdrawn from a cold bottle into an iced syringe and placed immediately in the center of the tumor by hypodermic needle. Sublethal injections of 0.001 to 0.002 cc. per kilogram of this substance were given over a period of one week and while the animals appeared somewhat toxic and dizzy, no effects were noted upon the tumor growth during the time of injection and upon post-mortem examination no more necrosis was observed in these tumors than in untreated tumors of a similar size.

Following the recent publication by Schubert[2] regarding compounds of thiolacids with aldehydes, it was felt that a combination of cysteine and formaldehyde might prove to be an effective chemical for tumor control. Accordingly, the compound was made in the manner described by Schubert and after purification was injected into Jensen's sarcoma in rats. Thirty-three mg. of this substance were injected into sarcoma nodules in 150-gm. white rats for 5 successive days. In none of these animals were there any appreciable effects on tumor growth and in none of them either during life or at post-mortem was there any apparent tumor necrosis. Subsequent experiments with homocysteine, glutathione and other sulphur-bearing amino acids are being tried and will be reported. Cystine has given uniformly negative results. Cysteine hydrochloride, on the other hand, has been consistent in causing necrosis and regression in the Jensen rat sarcoma. Studies on the effects of cysteine hydrochloride upon human tumors are in progress.

[2] Schubert, M. P., *J. Biol. Chem.*, 1936, **114**, 341.

8967 P

Regression Immunity to Jensen's Sarcoma After Cysteine Injection

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The production of immunity to subsequent inoculations of Jensen's sarcoma was described.[1] To determine the duration of this immunity, white rats, inoculated with tumor which subsequently regressed following injections of cysteine hydrochloride, have been inoculated with fresh emulsions of Jensen's sarcoma each month since February 1, 1936. Inoculations have been attempted once a month for 7 months on each of 8 rats immunized by the regression of the tumors 7 months previously. In no case has there been an acceptance of the inoculation by the animal, all being consistently immune to this particular type of sarcoma. In a group of 4 other animals immunized at this same time by the regression of Jensen's sarcoma, inoculations of the Emge sarcoma have also failed to take.

In order to ascertain the mass of tumor regression necessary to produce subsequent immunity, an experiment was devised in which 20 rats were inoculated with Jensen's sarcoma. Fifteen of these animals developed tumors. Seven to 9 days after inoculation, when the tumors in 5 of these rats were 1.0 cm. in diameter, they were injected with a single dose of 50 mg. of cysteine hydrochloride in 1 cc. water. Similar injections were made 11 to 14 days after inoculation in another group of 5 rats when the tumors had grown to 2.0 cm. in diameter, and in a third group of 5 rats, 18 to 24 days after inoculation, when the tumors were 3.0 cm. in diameter. Complete regression occurred within 14 days in all these injected tumors. Three attempts, at approximated 2-week intervals, from 3 weeks to 2 months after the cysteine injection, to reinoculate the Jensen rat sarcoma into each of these animals have failed. It is not feasible to inject tumor masses smaller than 1.0 cm. in diameter with cysteine because the tissue mass cannot be accurately distinguished as tumor since it may be a small area of infection or necrosis resulting from the attempted inoculation.

[1] Connor, C. L., Carr, J. L., and Ginzton, L. PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 374.

8968 C

Effect of Crystalline Synthetic Androsterone on the Female Bitterling.*

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We have reported that the lengthening of the ovipositor of the female bitterling† may be produced by that fraction of male urine containing male hormone and have suggested[1] this biological reaction as a test for male hormones. We have recently been engaged in experiments with the crystalline synthetic male hormones. These materials are scarcely soluble in water and hence emulsions were first employed. Later the use of propylene glycol as a solvent was suggested by Dr. Warren M. Cox, Jr., of Mead Johnson & Co. This solvent in small amounts is in itself harmless to the fish and does not cause this reaction. The sterols dissolve in it with the aid of heat and when such solutions are added to the large volumes of water in which the fish are placed, the material remains as a fine suspension.

Under these conditions synthetic androsterone‡ was found to produce positive reactions when added in very small amounts. Like many other biological phenomena the reaction is not obtained in every instance. This may account for the recent negative report[2] of a single experiment on 2 fish. The reaction seems to occur more slowly with crystalline androsterone than with urine—possibly because the hormone is present in a more soluble form in urine. Therefore we have taken not only the 48-hour reading, which is ordinarily sufficient in this test, but also the 72-hour reading. The experiments were conducted in the same manner as previously reported.[1]

Our first experiments were performed in June when, as is well known, the fish are in a less reactive state. Doses of 1 and 2 mg. were ineffective while 4 and 6 mg. gave positive results.

* Aided by a grant from the Lucius N. Littauer Foundation.

† We are greatly indebted to Mr. Christopher W. Coates of the New York Aquarium for his cooperation.

1 Kleiner, I. S., Weisman, A. I., and Mishkind, D. I., *J. A. M. A.*, 1936, **106**, 1643.

‡ We wish to thank Schering & Co. and Dr. Erwin Schwenk for the synthetic androsterone used.

2 Barnes, B. O., Kanter, A. E., and Klawans, A. H., *Science*, 1936, **84**, 310.

After September 15, the fish were found to be in a normally reactive state and tests were resumed. Negative results were experienced with doses of 0.7 mg. or less. Between 0.8 and 1.2 mg. the results were always positive in 48 or 72 hours (4 experiments). With larger amounts (1.4-4 mg.) variable results were obtained, mostly negative in 48 hours, with 3 positive and 5 negative in 72 hours. The larger doses seem to have a depressing effect on the fish. They become rather sluggish and this may account for their failure to react to large doses. It is therefore evident that for crystalline synthetic androsterone a positive reaction depends on its dosage and state of suspension.

8969 C

Attempts to Infect the Common Marmoset Monkey with the Virus of Poliomyelitis.*

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From the Laboratories of the Infantile Paralysis Commission of the Long Island College of Medicine.

One of the serious drawbacks in experimental poliomyelitis has been the failure consistently to transmit the disease to any animal except the Rhesus monkey. This has limited the scope of investigations of this disease. However, recent success with other viruses in lower animals, has stimulated us to continue these efforts.[1]

Other attempts have been made to infect new world monkeys, without success. Flexner and Lewis,[2] Kraus and Kantor,[3] and Jungeblut and Engle[4] failed to transmit the disease to the Cebus monkey. Mackay and Schroeder[5] failed to infect the Spider monkey.

* This work was supported by grants from the Rockefeller Foundation, the Friedsam Foundation, and by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

1 Stuart-Harris, C. H., *Brit. J. Exp. Path.*, 1936, **17**, 324; Findlay, G. M., and Clarke, L. P., *Trans. R. Soc. Trop. Med. Hyg.*, 1934, **28**, 335; Theiler, M., *Ann. Trop. Med. and Parasit.*, 1930, **24**, 249; Webster, L. T., and Fite, G. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 656.

2 Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 45.

3 Kraus, R., and Kantor, L., *Rev. d. Inst. Bact.*, 1917, **1**, 43.

4 Jungeblut, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

5 Mackay, Eaton M., and Schroeder, Charles R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 373.

The Marmoset was selected because of its position in the monkey family tree, being the lowest in the sub-order of anthropoids. It was felt that should successful infection take place in this monkey, further transmission to still lower animals might be more readily accomplished; this perhaps making it possible to employ with safety, as in rabies, a "fixed" living virus for active immunization of human beings.

On July 24, 1936, 2 common Marmosets were inoculated with a 10% saline suspension of active poliomyelitic cord; 0.5 cc. was administered intracerebrally into each animal and 1.1 cc. intraperitoneally. Daily temperatures were taken and the animals closely observed for any signs of illness; no alterations in temperature or behavior were noted and following the technique of Flexner,[6, 7] the animals were reinoculated one week later, August 1, 1936), with the same material, dosage and route of administration as was used in the primary inoculation. After an observation period of 9 days, in which no changes of temperature or in the health of the monkeys were noted, the animals were again inoculated (August 10) with a 20% suspension of active poliomyelitic cord (0.5 cc. intracerebrally and 2.3 cc. intraperitoneally). A control Rhesus monkey, inoculated intracerebrally with 1.2 cc. of this suspension, developed typical poliomyelitis in 7 days. The Marmosets appeared unaffected by these inoculations.

Two additional animals each received 1 cc. of a 10% suspension of active poliomyelitic cord into each nostril on 3 successive days. A control Rhesus monkey, similarly treated, developed the frank experimental disease in 5 days. The 2 Marmosets remained unaffected.

On August 10, 1936, a fifth animal was inoculated by all 3 routes with a 20% suspension of the same batch of virus. Five-tenths of a cc. was administered intracerebrally, 2.1 cc. intraperitoneally, and 1 cc. was instilled into each nostril on 3 successive days. A normal Rhesus monkey similarly treated developed poliomyelitis in 7 days. The Marmoset remained unaffected and has remained well up to the present time, a period of over 3 weeks' observation.

Two of the Marmosets were reinoculated following the technique described by Sawyer and Lloyd.[8] On September 3, 1936, both animals were inoculated intracerebrally with 0.5 of a 2% solution of boiled starch in normal saline. About one minute later the animals received 0.5 cc. of a 10% suspension of active poliomyelitic

[6] Flexner, S., *Science*, 1931, **74**, 520.

[7] Flexner, S., *Science*, 1933, **77**, 413.

[8] Sawyer, W. A., and Lloyd, Wray, *J. Exp. Med.*, 1931, **54**, 533.

cord into the same intracerebral site and 3.1 cc. of the virus suspension was inoculated intraperitoneally. Both these animals have remained unaffected up to the present time, a period of 19 days.

Summary and Conclusions. Five common Marmoset monkeys were inoculated intracerebrally, intraperitoneally, and intranasally with 10 and 20% suspensions of active poliomyelitic cord. The monkeys remained unaffected by such inoculations. The common Marmoset does not therefore appear to be susceptible to infection with the virus of poliomyelitis.

8970 P

Reduced Ascorbic Acid Content of Blood Plasma in Rheumatoid Arthritis.*

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From the Departments of Pathology and the Arthritis Clinic, University of California Medical School.

Experimental, clinical and other considerations led to the concept that vitamin C deficiency may operate as a contributory factor in the etiology of some cases of rheumatoid arthritis.[1]

The present study, based upon the determination of reduced ascorbic acid in the blood plasma, represents an effort to evaluate objectively the validity of this thesis. The report of Abt, Farmer and Epstein[2] and our own,[3] indicate that the method proposed by Farmer and Abt[4] is accurate and a reliable index of the intake of vitamin C in health and in any case of the immediate nutritive status with respect to the vitamin.

On the basis of excretion tests and a comparative study of diet habit and reduced ascorbic acid determinations in a group of "normal" adults,[3] we feel that fasting plasma levels below 0.7 mg. per 100 cc. are probably sub-optimal. Levels ranging between 0.7 and 0.9 mg. per 100 cc. would seem adequate. Optimal levels probably lie

* This work was aided by a donation from the California Fruit Growers Exchange and by the Christine Breon Fund for Medical Research. We are indebted to Hoffmann-La Roche, Inc., for supplies of ascorbic acid.

1 Rinehart, J. F., *Ann. Int. Med.*, 1935, **9**, 671.

2 Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Pediat.*, 1936, **8** 1.

3 Greenberg, L. D., Rinehart, J. F., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 135.

4 Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

above this range. Reduced ascorbic acid levels below 0.5 mg. per 100 cc. must be considered low. These ranges are somewhat less than those recorded by Abt, Farmer and Epstein.[2] It is pertinent to recall that the estimation of the plasma ascorbic acid is only a measure of the immediate nutritive level, and is dependent upon recent dietary habit to a great degree. Although it is an index of vitamin C nutrition at the time of the test, in a single case a low level does not imply tissue injury or sub-clinical scurvy. This latter results from the operation of sub-optimal or low metabolic levels over some period of time. Conversely, a good or high level would not indicate that deficiency had not operated to produce tissue injury in the past.

The material of this study comprises 36 cases of rheumatoid arthritis. Twelve of these cases serve as a control group in that they had all been maintained for a period of months on a high vitamin C intake. The plasma ascorbic acid levels in this group ranged from 0.90 to 1.39 mg. per 100 cc. with an average of 1.10 mg. This, no doubt, approaches an optimal range.

A second group of 21 cases all showed clinical or laboratory evidence of activity of the rheumatic process. The fasting plasma ascorbic acid levels in this group ranged from 0.14 to 0.66 mg. per 100 cc. They are shown graphically in Fig. 1.† In 5 of the cases a high vitamin C intake had been recommended at some time in the past but for one reason or another the patients had not cooperated to the extent advised (cases stippled in graph). Three other cases

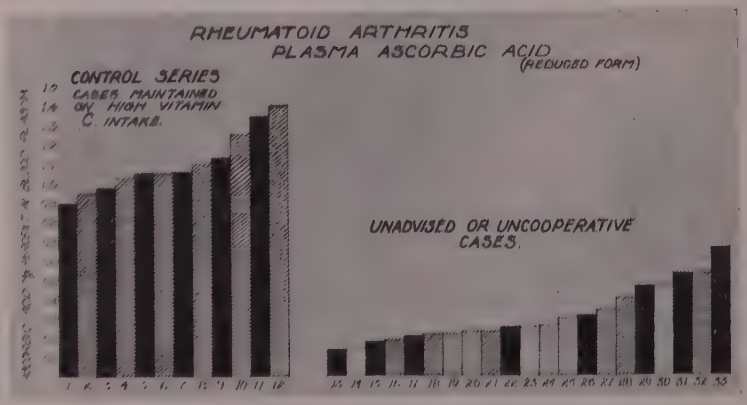

FIG. 1.

† The single value above 0.53 mg. did not represent the habitual nutritive level of the patient because she had been on a raw fruit and vegetable diet for 3 weeks prior to the determination.

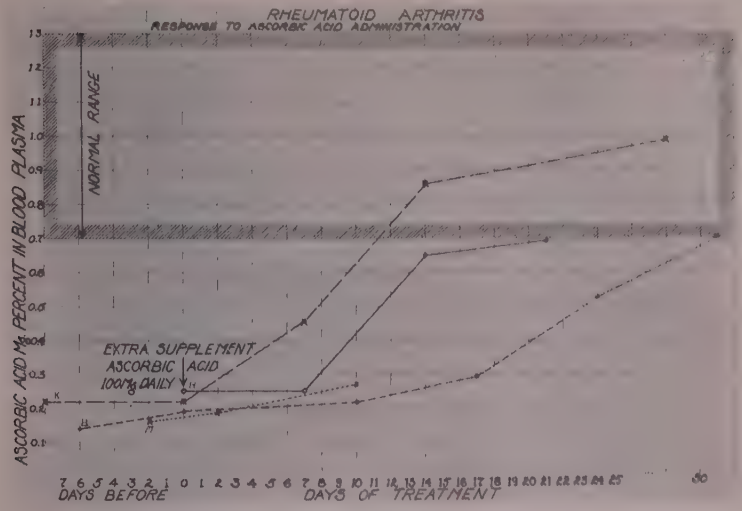

FIG. 2.

are of particular interest in that they had taken a good though not high vitamin C diet supplement for months prior to the examination (cases unshaded in graph). In spite of this the ascorbic acid plasma levels were sharply lowered. Such cases strongly suggest that a fault in absorption or utilization may be the basis for vitamin C under-nutrition in some individuals. In the majority of the cases, definite vitamin C supplements were prescribed following initial observations. In all of the cases which we were able to follow, the reduced ascorbic acid plasma levels rose in response to this regime. The initial levels and responses to therapy in 4 cases are shown in Fig. 2. This graph shows not only the depressed initial levels in rheumatoid arthritis, but the response of the plasma ascorbic acid concentration to an increased vitamin C intake. The refractory responses are to be noted in curves "M" and "B." Curve "H" is of one who had been on a relatively good vitamin C intake for several months (average supplement of 6 oz. of orange or tomato juice daily).

Three cases of old inactive rheumatoid arthritis gave reduced ascorbic acid plasma levels of 0.59 and 0.79 and 1.56 mg. per 100 cc.

The 6 cases of hypertrophic arthritis, in which plasma ascorbic acid was determined, showed high levels ranging from 0.90 to 1.34 mg. per 100 cc.

Lowered plasma ascorbic acid levels are obviously not peculiar to rheumatoid arthritis and, as has been noted, in individual cases, do

not establish the existence of scurvy. The practically uniform finding of sharply lowered levels in initial determinations in a series of 21 cases is, however, considered significant. With the exception of the 5 cases noted these individuals were on their usual dietary regime.

Summary. The blood plasma ascorbic acid (reduced form) in active cases of rheumatoid arthritis is regularly low if the individuals have not been maintained on a *high* vitamin C supplement. Unadvised cases were found to show uniformly lowered levels. The reduction is striking. Such levels rise in response to extra supplements of vitamin C. In many this rise is refractory. Our studies indicate that in some cases the intake required to maintain adequate vitamin C levels in the plasma are much above the average requirement for normal individuals. The mechanism involved is unexplained.

8971 P

Reduced Ascorbic Acid Content of Blood Plasma in Rheumatic Fever.*

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The thesis was advanced that vitamin C deficiency may be an important factor in the etiology of rheumatic fever.[1] This concept was based upon the experimental production of a disease state with manifold similarities to rheumatic fever, by subjecting guinea pigs to the combined influence of vitamin C deficiency and infection. Epidemiologic and clinical considerations were noted which afforded confirmatory evidence for the validity of the concept. In studies reported by Schultz, Sendroy and Swift,[2] and Perry[3], the clinical significance of this concept was questioned or denied.

* Aided by a donation from the California Fruit Growers Exchange and by the Christine Breon Fund for Medical Research. We are also indebted to Hoffmann-LaRoche, Inc., for supplies of ascorbic acid.

[1] Rinehart, J. F., and Mettier, S. R., *Am. J. Path.*, 1934, **10**, 61; Rinehart, J. F., Connor, C. L., and Mettier, S. R., *J. Exp. Med.*, 1934, **59**, 97; Rinehart, J. F., *Ann. Int. Med.*, 1935, **9**, 586; Rinehart, J. F., *J. Lab. and Clin. Med.*, 1936, **21**, 597.

[2] Schultz, M. P., Sendroy, J., and Swift, H. F., *J. Clin. Invest.*, 1935, **14**, 698.

[3] Perry, C. B., *The Lancet*, 1935, **229**, 426.

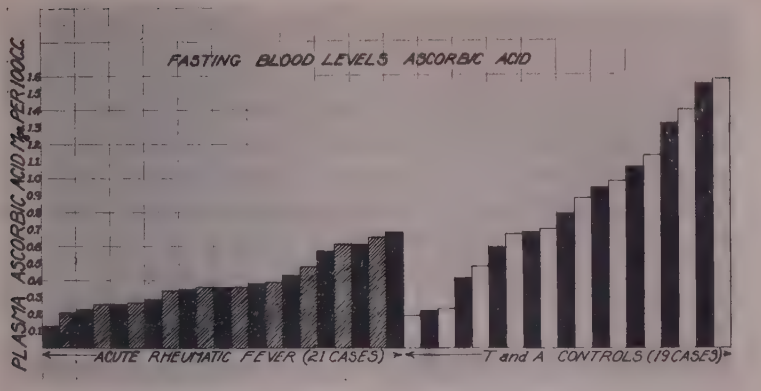

FIG. 1.

The present communication is based upon the study of the fasting blood plasma levels of reduced ascorbic acid in cases of rheumatic fever and chorea along with control groups. This and current studies[4] indicate that the plasma level of reduced ascorbic acid is an accurate index of the vitamin C intake as recently reported by Farmer and Abt.[5] The ascorbic acid plasma levels in our control groups are somewhat lower than those of Farmer and Abt, whose method we employed, probably because our study is based on fasting blood specimens. This, we feel, is an essential precaution for adequate comparative data.

In 21 cases of acute rheumatic fever the reduced ascorbic acid content of the blood ranged from 0.13 to 0.68 mg. per 100 cc., with an average of 0.39 mg.† The distribution is shown in the accompanying diagram (Fig. 1). A control series of cases, composed of children admitted to the University of California Hospital for tonsillectomy, is shown for comparison. In this group of 19 cases the range was 0.22 mg. % to 1.57 mg. % with an average of 0.81 mg. %. This series represent a comparable social and age group.‡

[4] Greenberg, L. D., Rinehart, J. F., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 135.

[5] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

† Three of the 5 cases with levels above 0.5 mg. % were not seen before the vitamin C content of the diet had been materially increased.

‡ At the time this study was reported data on a significant number of cases with infections, other than rheumatic fever, were not available. At present ascorbic acid determinations have been made on 19 cases of miscellaneous non-rheumatic infections in children. In this group, plasma values range from 0.16 to 1.61 mg. per 100 cc. with an average of 0.78 mg. 74% of the cases gave values above 0.5 mg. per 100 cc.

Five children who had suffered rheumatic fever in the past and had been maintained on a controlled high vitamin C intake for months prior to the examination, showed ascorbic acid levels ranging from 0.84 mg. % to 1.15 mg. %, with an average of 1.02 mg. %. This probably approaches an optimal post absorptive level. Seven cases, quiescent at the time of examination but with a history of recent or old rheumatic activity, gave values which ranged from 0.44 mg. % to 0.71 mg. %; averaging 0.53 mg. %. Three other cases of chronic rheumatic heart disease for whom a high vitamin C intake had been advised gave levels of 0.37, 0.45, and 0.67 mg. %. Poverty precluded satisfactory cooperation in this group. These data indicate that "rheumatic" children (although not suffering from active disease), unless maintained on a controlled high vitamin C intake, are likely to lie in a sub-optimal metabolic range with respect to vitamin C.

In the majority of cases of acute rheumatic fever, after basal observations, vitamin C was supplied in generous amounts and subsequent determinations made. The vitamin C was given as orange juice or as ascorbic acid. The dosage, in terms of ascorbic acid, ranged from 100 mg. to 500 mg. daily. In cases that could be followed for adequate periods the plasma levels of ascorbic acid rose following this regime. In some instances this occurred rather

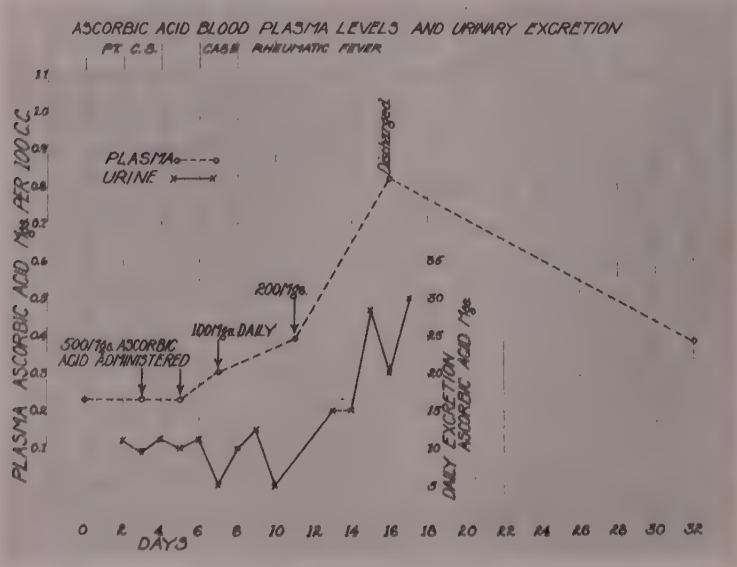

FIG. 2.

promptly; in others the rise was delayed. A particularly refractory case, in which we had the opportunity to study both blood plasma levels and urinary excretion, is shown in Fig. 2. The failure of increased urinary excretion and the slight elevation of the plasma ascorbic acid after 2 massive doses of vitamin C is shown. The fall of the plasma level after the patient was discharged from the hospital is significant. Another case of unusual interest was that of an 11-year-old child who suffered from a very severe acute rheumatic carditis. In spite of massive doses of vitamin C, ranging from 200 to 400 mg. daily, the plasma ascorbic acid remained depressed for a period of 40 days. This case terminated fatally.

It is worthy of note that cases of uncomplicated chorea did not show the uniformly depressed plasma ascorbic acid levels that occurred in rheumatic fever. Four cases gave initial fasting blood levels of 0.27, 0.94, 1.0 and 1.18 mg. ascorbic acid per 100 cc.

A concurrent study[4] leads us to believe that fasting plasma ascorbic acid levels below 0.7 mg. per 100 cc. lie in a sub-optimal range. This is in essential agreement with the findings of Abt, Farmer, and Epstein.[6] They found ascorbic acid levels ranging from 0.8 to 2.0 in a group of infants and children on adequate diets. A group on low vitamin C intake ranged from 0.51 to 0.77 mg. per 100 cc.

Summary. In acute rheumatic fever the reduced ascorbic acid level of the blood plasma is found to be almost uniformly lowered. Our data further indicates that at least some and perhaps a high percentage of "rheumatic" children, although the disease process is clinically quiescent, tend to lie in less low but distinctly sub-optimal ranges. It has not been determined that this is due to inadequate intake or to what extent it may have resulted from anorexia, digestive disorders or depletion by the disease itself. The data presented indicates that the plasma ascorbic acid levels usually parallel the vitamin C intake. However, some cases are quite refractory in their responses to oral administration of the vitamin even in large doses.

[6] Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Pediat.*, 1936, **8**, 1.

8972 C

Influence of Gonads on Exophthalmos in Rabbits.

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The chronic progressive exophthalmos of the type seen in Graves' disease, in its uncomplicated form, is dependent upon a highly complex, though probably specific, disturbance in the balance of internal secretions which causes central stimulation of the sympathetic innervation of the muscles of Müller. It has been shown that thyroid insufficiency (either because of goiter or thyroidectomy) strikingly increases the incidence and shortens the time of production of exophthalmos in rabbits and guinea pigs.[1-5] The increase of postoperative exophthalmos in Graves' disease during the last decade is probably of the same nature. Feeding desiccated thyroid prevents it and often cures it in rabbits and guinea pigs. Thyroid insufficiency, relative or absolute, therefore, is a necessary condition in order that this form of exophthalmos may develop. Yet it does not occur in the severe thyroid insufficiencies of endemic cretinism in man, nor in spontaneous Gull's disease (*i. e.*, not preceded by Graves' disease). It is obvious that other factors than thyroid insufficiency are involved. Sex and age differences have been mentioned in earlier papers and in this report we shall present data relative to these factors.

In rabbits under 3 months and over 7 months old we have rarely been able to obtain frank exophthalmos, although maintained on a diet of alfalfa hay and oats and given daily intramuscular injections of methyl cyanide. Exophthalmos develops most frequently in puberal rabbits (4-5 months). In a general way this age factor is also seen in the exophthalmos of Graves' disease. Under our experimental conditions during the past 4 years rabbits between the ages of 3 and 7 months have shown the following incidence of exophthalmos:

1. In rabbits with intact thyroids, 63 males out of 131, or 48 %, developed frank exophthalmos; while 37 females out of 125, or

[1] Marine, D., Baumann, E. J., Spence, A. W., and Cipra, A., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 822.

[2] Marine, D., Rosen, S. H., and Cipra, A., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 649.

[3] Marine, D., and Rosen, S. H., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 901.

[4] Marine, D., and Rosen, S. H., *Am. J. Med. Sci.*, 1934, **188**, 565.

[5] Smelser, G. K., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 128.

30%, developed exophthalmos. Thus under our conditions the incidence was 18% greater in males.

2. In thyroidectomized rabbits, 47 males out of 82, or 57%, developed exophthalmos; while 19 females out of 41, or 46%, developed exophthalmos.

Thyroidectomy clearly steps up the incidence of exophthalmos but does not greatly, if at all, influence the sex difference. A similar sex difference appears in postthyroidectomy exophthalmos in Graves' disease. Thus, of 52 cases reported in the recent literature, 31, or 60%, were males and 21, or 40%, were females (data of the percentage incidence of exophthalmos in non-operated male and female cases of Graves' disease are not available.) In these human cases the average age was 53 years for males and 42 for females.

Therefore, other factors beside thyroid insufficiency and age are involved in the production of exophthalmos. We have already pointed out that those rabbits which develop the best exophthalmos also were sexually more active or frankly precocious. In the light of our present knowledge this would be explained by assuming that the intense stimulation of the anterior pituitary which follows thyroidectomy brought about the stimulation of the gonads. In view of the sex and age differences and the increased sexual development following thyroidectomy in puberal rabbits, we have studied the effect of gonadectomy in rabbits on the development of exophthalmos. Thirty-eight normal young adult male and 23 female rabbits were gonadectomized. All survived more than 2 months. No frank case of exophthalmos developed, where in a similar group without gonadectomy 50% of the males and 30% of the females should have developed exophthalmos. Two males and 2 females developed slight exophthalmos (+?) during the second and third weeks after gonadectomy, but these receded to —? at the end of the fifth week. In 3 rabbits with frank exophthalmos at the time of gonadectomy, the exophthalmos receded after gonadectomy. These experiments indicate that functionally active gonads in the rabbit greatly increase the incidence of exophthalmos and that failure of development and decline in gonadal activity may partially explain the absence of exophthalmos in cretinism and Gull's disease. What particular function of the gonads is involved is not known.

Oestrone (menformon, theelin) in doses of 100 rat units twice daily for 2 months has not influenced the existing exophthalmos in 2 male rabbits. Pituitrin-S (0.25 cc. twice daily for 12 days intramuscularly) was given to 6 rabbits without effect, and adrenalin (Parke, Davis 1-1000), beginning with 0.2 cc. and gradually

increasing to 2 cc. twice daily intramuscularly, was given to 22 rabbits over a period of 2 months without producing, or modifying existing, exophthalmos.

Summary. Male rabbits develop exophthalmos more frequently than females. This difference is independent of the thyroid gland. Exophthalmos develops most frequently in rabbits about the age of puberty (4-5 months). Gonadectomy greatly reduces the incidence even in thyroidectomized rabbits. Oestrone (menformon, theelin), pituitrin-S and adrenalin in the dosages and method of administration used neither produce, nor modify existing, exophthalmos.

8973 P

Prevention of Atherosclerosis in Rabbits. I. Administration of Potassium Thiocyanate.

WILLIAM MARIAS MALISOFF. (Introduced by D. Marine.)

From the Laboratory Division of the Montefiore Hospital, New York.

Many efforts have been made to counteract the atherosclerosis produced in rabbits by the feeding of cholesterol or cholesterol-containing foods with or without thyroidectomy.[1] These efforts have converged on a demonstration that iodides may to an extent replace the loss of thyroid in retarding the development of atheromata.[2-4] Completely negative results were reported for bromides. Otherwise attempts to get effects with organic compounds, *e. g.*, chlorophyll and alcohol, have either been unsubstantiated when reported positive, or gave no conclusive results.

Good evidence exists as to the serious changes in permeability of arterial walls following both thyroidectomy and the feeding of cholesterol.[5] It seemed reasonable, therefore, to follow out the investigations suggested by the use of ions. The Hofmeister series, although inaccurate in detail, unmistakably points to an ion which would be expected to be more effective than iodide, namely thiocyanate. At the same time it explains the ineffectiveness of the bromide ion. The relatively low toxicity of thiocyanates, coupled

[1] Anitschkow, N., in *Arteriosclerosis*, Macmillan, New York, 1933.
[2] Ungar, H., *Arch. Exp. Path.*, 1934, **175**, 536.
[3] Turner, K. B., *J. Exp. Med.*, 1933, **58**, 115.
[4] Turner, K. B., and Khayat, G. B., *J. Exp. Med.*, 1933, **58**, 127.
[5] Duff, G. L., *Arch. Path.*, 1935, **20**, 81, 259.

with the fact that they are to an extent normal constituents of body fluids, led to the following procedure.

Twelve unselected young adult female rabbits from a strain which has been used in this laboratory for 12 years were thyroidectomized. A group of 4, which served as controls, were fed approximately 0.3 gm. cholesterol per kg. per day; a second group of 4 received the same plus 20 mg. per kg. per day of potassium thiocyanate; a third group received the same plus 60 mg. per kg. per day of potassium thiocyanate. All rabbits were given a 2.5% solution of calcium lactate as drinking water for 5 days.

The cholesterol was a very pure product of the Wilson Co., Chicago. The KCNS was recrystallized 2 times from an initially pure product to ensure freedom from traces of impurities, such as iodides. The diet consisted of alfalfa hay and oats with occasional greens.

Examinations were made of the principal organs, especially the aorta and the kidneys. Some blood cholesterols were determined.

Table I shows the results with respect to the appearance of cholesterol in the aorta and kidneys.

TABLE I.

Changes in Rabbits' Aortas and Kidneys on Feeding Cholesterol with and without Potassium Thiocyanate.

Wt.		KCNS mg. per kg. per day	No. of Days	Aorta	Kidneys	Thyroid at Autopsy	Blood Cholesterol
Start	Finish						
2261	2777	0	66	+	+	—	.254
1735	2032	0	59	+++	+++	—	
2036	2371	0	61	+++	+++	—	.530
1765	1800	0	52	+++	+++	tiny fragment	
1776	2096	20	61	+	++	—	
1972	2183	20	52	—	+	—	
1712	1754	20	59	++	+++	—	
2133	2580	20	66	—	+	2 x 2 mm.	.894
2067	2653	60	66	—	—	two 2 x 2 mm.	.387
2110	2354	60	61	—	—	tiny fragment	
1803	2165	60	56	—	—	—	
1946	1581	60	49	—	—	tiny fragment	

The amount deposited is judged as +, ++, +++, and absence as —.

The data present a picture of protection of the aorta by moderate amounts of potassium thiocyanate. Under the conditions the minimal dose must lie somewhere between 20 and 60 mg. of thiocyanate per kilogram body weight for a period of approximately 2 months. Grossly considered the protection seems to extend to the kidneys as well, but is only clear-cut in the case of the larger dosage of potassium thiocyanate.

The presence of tiny fragments of thyroid in 3 cases is considered irrelevant to the results. In 2 cases larger amounts of thyroid tissue, but still very small, were found, but they too are deemed insufficient to affect the results (*Cf.* Turner and Khayat, *loc. cit.*). Besides, the rabbits involved had a high cholesterolemia.

In the case of the control animals the largest rabbit showed slight deposition only. This may be attributed to some extent to the fact that it was receiving relatively about 2/3 of the dosage of cholesterol of the other animals. An analysis of its blood cholesterol showed only 0.254 as against 0.894, 0.530, and 0.387 in other animals. Nevertheless there may be other factors at play.

Whether the protection would break down in a longer period of time, or whether the administration of potassium thiocyanate following the development of atheromata would remove them, are problems reserved for further study. The thiocyanate ion may serve to heighten the degree of dispersion of the cholesterol colloids and it may have a distinct effect on the permeability of the arterial walls, or both. These possibilities, however, are to be investigated separately. Similarly further progress is visioned along the lines of extending the findings to other animals, closer to man in their dietary habits.

Conclusions. Potassium thiocyanate exercises a protective action against the development of cholesterol atherosclerosis in thyroidectomized rabbits.